DOCUMENT-IDENTIFIER: US 6328885 B1
TITLE: Current-efficient suppressors

DEPR:
The term "packing" refers to stationary flow-through solid material disposed in a flow channel of the suppressor. It can be a screen or a porous **monolithic** matrix, a resin particle bed or other form. It can be strongly charged, weakly charged or of neutral charge, as will be explained. The term packing is alternatively called "bridging means."

DEPR:
In the above system, one way to increase current efficiency is leave the sample stream flow channel open without packing or to use packing which is of neutral charge or of low capacity relative to the packing of high capacity ion exchange material in the ion receiving flow channel and, for a two membrane suppressor, in the ion source channel. While the above description refers to the stationary flow-through packing of ion exchange material in the form of a high capacity charged screen, other forms of packing may also be employed as described above. Such other packing forms of ion exchange material include packed beds of ion exchange resin or **monolithic** materials of charged material with sufficient porosity for the flow of an aqueous liquid stream through them. The packing in the ion receiving channel has a substantially higher capacity than ion exchange packing in the sample flow channel, if present. Thus, if a charged packing is used in the sample stream flow channel, it preferably is of low capacity, with a capacity of substantially less than that of the packing in the ion receiving flow channel. Suitably, the ratio of total capacities of the packing in the sample stream flow channel to that in the ion receiving stream

flow channel is no greater than about 0.9, and preferably no greater than about
0.7 to 0.5, and more preferably no greater than about 0.1.

CCOR:
210/198.2

DOCUMENT-IDENTIFIER: US 6325976 B1
TITLE: Continuous electrolytically regenerated packed bed suppressor for ion
chromatography

CCXR:
210/198.2

ORPL:
Petro et al., "Molded **Monolithic** Rod of Macroporous Poly(styrene-co-divinybenzene) as a Separation Medium for HPLC of Synthetic
Polymers: "On-Column" Precipitation -Redissolution Chromatography as an
Alternative to Size Exclusion Chromatography of Styrene Oligomers an dPolymers"
Analytical Chemistry, 68(2):315-321 (Jan. 15, 1996).

DOCUMENT-IDENTIFIER: US 6309549 B1
TITLE: Polynucleotide separations on polymeric separation

ABPL:
Non-polar polymeric separation media, such as beads or **monoliths,** are suitable
for chromatographic separation of mixtures of polynucleotides when the surfaces
of the media are unsubstituted or substituted with a hydrocarbon group having
from one to 1,000,000 carbons and when the surfaces are substantially free from
multivalent cation contamination. The polymeric media provide efficient
separation of polynucleotides using Matched Ion Polynucleotide Chromatography.
Methods for maintaining and storing the polymeric media include treatment with
multivalent cation binding agents.

BSPP:
The present invention is directed to the separation of polynucleotides using
non-polar separation surfaces, such as the surfaces of polymeric beads and
surfaces within molded **monoliths,** which are substantially free from
contamination with multivalent cations.

BSPP:
Another object of the present invention is to provide a method for separating
polynucleotides using nonporous polymer separation media, such as beads or
monoliths (e.g., rods), having non-reactive, non-polar surfaces.

BSPP:
In the [illegible] is a method for separating a mixture of
[illegible] up to 1500 base
pairs in a polymeric separation medium having non-polar surfaces which are
substantially free from contamination with multivalent cations, and eluting the
mixture of polynucleotides. The preferred surfaces are nonporous. The

non-polar surfaces can be enclosed in a column. In the preferred embodiment, precautions are taken during the production of the medium so that it is substantially free of multivalent cation contaminants and the medium is treated, for example by an acid wash treatment and/or treatment with multivalent cation binding agent, to remove any residual surface metal contaminants. The preferred separation medium is characterized by having a DNA Separation Factor (defined hereinbelow) of at least 0.05. The preferred separation medium is also characterized by having a Mutation Separation Factor (as defined hereinbelow) of at least 0.1. In the preferred embodiment, the separation is made by Matched Ion Polynucleotide Chromatography (MIPC, as defined hereinbelow). Examples of non-polar surfaces include the surfaces of polymer beads and the surfaces of interstitial spaces within a polymeric monolith. The elution step preferably uses a mobile phase containing a counterion agent and a water-soluble organic solvent. Examples of a suitable organic solvent include alcohol, nitrile, dimethylformamide, tetrahydrofuran, ester, ether, and mixtures of one or more thereof, e.g., methanol, ethanol, 2-propanol, 1-propanol, tetrahydrofuran, ethyl acetate, acetonitrile. The most preferred organic solvent is acetonitrile. The counterion agent is preferably selected from the group consisting of lower alkyl primary amine, lower alkyl secondary amine, lower alkyl tertiary amine, lower trialkyammonium salt, quaternary ammonium salt, and mixtures of one or more thereof. Non-limiting examples of counterion agents include hexylammonium acetate, [illegible] acetate, decylammonium acetate, [illegible] acetate, pyridiniumammonium acetate, cyclonexylammonium acetate, diethylammonium acetate, propylethylammonium acetate, propyldiethylammonium acetate, butylethylammonium acetate, methylhexylammonium acetate,

tetramethylammonium
acetate, tetrapropylammonium acetate, tetrabutylammonium acetate, dimethydiethylammonium acetate, triethylammonium acetate, tripropylammonium
acetate, tributylammonium acetate, tetraethylammonium acetate, tetrapropylammonium acetate, tetrabutylammonium acetate, and mixtures of any
one or more of the above. The counterion agent includes an anion, e.g.,
acetate, carbonate, bicarbonate, phosphate, sulfate, nitrate, propionate,
formate, chloride, perchlorate, or bromide. The most preferred counterion
agent is triethylammonium acetate or triethylammonium hexafluoroisopropyl
alcohol.

BSPR:
In yet another embodiment, the invention is a method for separating a mixture
of polynucleotides comprising flowing a mixture of polynucleotides having up to
1500 base pairs through a polymeric **monolith,** and separating the mixture of
polynucleotides using MIPC. In this embodiment, the non-polar separation
surfaces are the surfaces of interstitial spaces of a polymeric **monolith**. An
example of such a **monolith** is a polymeric rod prepared within the confines of a
chromatographic column. The **monolith** of the invention is characterized by
having a DNA Separation Factor of at least 0.05. In a preferred embodiment,
the **monolith** is characterized by having a DNA Separation Factor of at least
0.5. The **monolith** is preferably characterized by having a Mutation Separation
Factor of at least 0.1. The mobile phase used in the separation preferably
includes an organic solvent as exemplified by alcohol, nitrile, dimethylformamide, tetrahydrofuran, ester, ether, and mixtures thereof.
Examples of suitable solvents include methanol, ethanol, 2-propanol,
1-propanol, tetrahydrofuran, ethyl acetate, acetonitrile, and mixtures thereof.
The most preferred organic solvent is acetonitrile. The mobile phase
preferably includes a counterion agent such as lower primary,

secondary and
tertiary amines, and lower trialkyammonium salts, or quatemary ammonium salts.
More specifically, the counterion agent can be octylammonium acetate,
octadimethylammonium acetate, decylammonium acetate,
octadecylammonium acetate,
pyridiniumammonium acetate, cyclohexylammonium acetate,
diethylammonium
acetate, propylethylammonium acetate, propyldiethylammonium acetate,
butylethylammonium acetate, methylhexylammonium acetate,
tetramethylammonium
acetate, tetraethylammonium acetate, tetrapropylammonium acetate,
tetrabutylammonium acetate, dimethydiethylammonium acetate,
triethylammonium
acetate, tripropylammonium acetate, tributylammonium acetate,
tetrapropylammonium acetate, tetrabutylammonium acetate, and mixtures of any
one or more of the above. The counterion agent includes an anion, e.g.,
acetate, carbonate, bicarbonate, phosphate, sulfate, nitrate, propionate,
formate, chloride, perchlorate, and bromide. However, the most preferred
counterion agent is triethylammonium acetate.

BSPR:
In the preferred embodiment, precautions are taken during the production of the
polymeric **monolith** so that it is substantially free of multivalent cation
contaminants and the **monolith** is treated, for example, by an acid wash
treatment, to remove any residual surface metal contaminants. In the
embodiment, the **monolith** is characterized by having a DNA Separation Factor of
at least 0.05. In a preferred embodiment, the **monolith** is characterized by
having a DNA separation factor of at least 0.5. Also in a preferred
embodiment, the **monolith** is further characterized by having a Mutation Separation
Factor of at least 0.1.

BSPR:
In another aspect, the present invention is a method for treating the non-polar
surface of a polymeric medium used for separating polynuleotides

such as the
surface of beads in a MIPC column or the interstitial spaces in a polymeric
monolith, in order to improve the resolution of polynucleotides, such as dsDNA,
separated on said surface. This treatment includes contacting the surface with
a solution containing a multivalent cation binding agent. In a preferred
embodiment, the solution has a temperature of about 50.degree. C. to
90.degree. C. An example of this treatment includes flowing a solution
containing a multivalent cation binding agent through a MIPC column, wherein
the solution has a temperature of about 50.degree. C. to 90.degree. C. The
preferred temperature is about 70.degree. C. to 80.degree. C. In a preferred
embodiment, the multivalent cation binding agent is a coordination compound,
examples of which include water-soluble chelating agents and crown ethers.
Specific examples include acetylacetone, alizarin, aluminon, chloranilic acid,
kojic acid, morin, rhodizonic acid, thionalide, thiourea, .alpha.-furildioxime,
nioxime, salicylaldoxime, dimethylglyoxime, .alpha.-furildioxime, cupferron,
.alpha.-nitroso-.beta.-naphthol, nitroso-R-salt, diphenylthiocarbazone,
diphenylcarbazone, eriochrome black T, PAN, SPADNS, glyoxal-bis(2-hydroxyanil),
murexide, .alpha.-benzoinoxime, mandelic acid, anthranilic acid,
ethylenediamine, glycine, triaminotriethylamine, thionalide, triethylenetetramine, ethylenediaminetetraacetic acid (EDTA), metalphthalein,
arsinic acids, .alpha.,.alpha.'-bipyridine, 4-hydroxybenzothiazole,
8-hydroxyquinaldine, 8-hydroxyquinoline, 1,10-phenanthroline, picolinic acid,
quinaldinic acid, .alpha.,.alpha.',.alpha."-terpyridyl, [illegible], pyrocatechol, salicylic acid, tiron,
4-chloro-1,2-dimercapto benzene, urithiol, mercaptobenzothiazole, rubeanic acid,
oxalic acid, sodium diethyldithiocarbamate, and zinc dibenzyldithiocarbamate. However, the most preferred chelating agent is EDTA.
In this aspect of the invention, the solution preferably includes

an organic
solvent as exemplified by alcohol, trile, dimethyoformamide, tetrahydrofuran,
ester, ether, and mixtures thereof. Examples of suitable solvents include
methanol, ethanol, 2-propanot, 1 -ropanol, tetrahydrofuran, ethyl acetate,
acetonitrile, and mixtures thereof. he most preferred organic solvent is
acetonitrile. In one embodiment, the solution can include a counterion agent
such as lower primary, secondary and tertiary amines, and lower trialkyammonium
salts, or quaternary ammonium salts. More specifically, the counterion agent
can be octylammonium acetate, octadimethylammonium acetate, decylammonium
acetate, octadecylammonium acetate, pyridiniumammonium acetate, cyclohexylammonium acetate, diethylammonium acetate, propylethylammonium
acetate , propyldiethylammonium acetate, butylethylammonium acetate,
methylhexylammonium acetate, tetramethylammonium acetate, tetraethylammonium
acetate, tetrapropylammonium acetate, tetrabutylammonium acetate, dimethydiethylammonium acetate, triethylammonium acetate, tripropylammonium
acetate, tributylammonium acetate, tetrapropylammonium acetate, tetrabutylammonium acetate, and mixtures of any one or more of the above. The
counterion agent includes an anion, e.g., acetate, carbonate, bicarbonate,
phosphate, sulfate, nitrate, propionate, formate, chloride, perchlorate, and
bromide. However, the most preferred counterion agent is triethylammonium
acetate.

BSPR:
In yet a further aspect, the invention provides a method for storing a medium
used for separating polynucleotides, e.g., the beads of a MIP or a
polymeric monolith, in order to improve the resolution of double stranded DNA
fragments separated using the medium. In the case of a MIP column, the
preferred method includes flowing a solution containing a multivalent cation
binding agent through the column prior to storing the column. In

a preferred
embodiment, the multivalent cation binding agent is a
coordination compound,
examples of which include water-soluble chelating agents and
crown ethers.
Specific examples include acetylacetone, alizarin, aluminon,
chloranilic acid,
kojic acid, morin, rhodizonic acid, thionalide, thiourea,
.alpha.-furildioxime,
nioxime, salicylaldoxime, dimethylglyoxime, .alpha.-furildioxime,
cupferron,
.alpha.-nitroso-.beta.-naphthol, nitroso-R-salt,
diphenylthiocarbazone,
diphenylcarbazone, eriochrome black T, PAN, SPADNS,
glyoxal-bis(2-hydroxyanil),
murexide, a-benzoinoxime, mandelic acid, anthranilic acid,
ethylenediamine,
glycine, triaminotriethylamine, thionalide, triethylenetetramine,
EDTA,
metalphthalein, arsonic acids, .alpha.,.alpha.'-bipyridine,
4-hydroxybenzothiazole, 8-hydroxyquinaldine, 8-hydroxyquinoline,
1,10-phenanthroline, picolinic acid, quinaldic acid,
.alpha.,.alpha.',.alpha."-terpyridyl,
9-methyl-2,3,7-trihydroxy-6-fluorone,
pyrocatechol, salicylic acid, tiron,
4-chloro-1,2-dimercaptobenzene, dithiol,
mercaptobenzothiazole, rubeanic acid, oxalic acid, sodium
diethyldithiocarbarbamate, and zinc dibenzyldithiocarbamate.
However, the most
preferred chelating agent is EDTA. In this aspect of the
invention, the
solution preferably includes an organic solvent as exemplified by
alcohols,
nitriles, dimethylformamide, tetrahydrofuran, esters, and ethers.
The most
preferred organic solvent is acetonitrile. The solution can also
include a
counterion agent such as lower primary, secondary and tertiary
amines, and
lower trialkyammonium salts, or quatemary ammonium salts. More
particularly,
the counterion agent can be octylammonium acetate,
dioctylammonium
acetate, decylammonium acetate, dodecylammonium acetate,
pyridinium acetate,
acetate, cyclohexylammonium acetate, diethylammonium acetate,
propylethylammonium acetate, propyldiethylammonium acetate,
butylethylammonium
acetate, methylhexylammonium acetate, tetramethylammonium
acetate,
tetraethylammonium acetate, tetrapropylammonium acetate,

tetrabutylammonium
acetate, dimethydiethylammonium acetate, triethylammonium acetate,
tripropylammonium acetate, tributylammonium acetate, tetrapropylammonium
acetate, tetrabutylammonium acetate, and mixtures of any one or more of the
above. The counterion agent includes an anion, e.g., acetate, carbonate,
bicarbonate, phosphate, sulfate, nitrate, propionate, formate, chloride,
perchlorate, and bromide. However, the most preferred counterion agent is
triethylammonium acetate.

DEPR:
The medium can be enclosed in a column. In one embodiment, the non-polar
surfaces comprise the surfaces of polymeric beads. In an alternative
embodiment, the surfaces comprise the surfaces of interstitial spaces in a
molded polymeric **monolith**. For purposes of simplifying the description of the
invention and not by way of limitation, the separation of polynucleotides using
nonporous beads, and the preparation of such beads, will be primarily described
herein, it being understood that other separation surfaces, such as the
interstitial surfaces of polymeric **monoliths**, are intended to be included
within the scope of this invention. **Monoliths** such as rods contain polymer
separation media which have been formed inside a column as a unitary structure
having through pores or interstitial spaces which allow eluting solvent and
analyte to pass through and which provide the non-polar separation surface.

DEER:
In another embodiment of the present invention, the separation medium can be in
the form of a polymeric **monolith** such as a rod like **monolithic** column. The
monolithic column is polymerized or formed as a single unit inside of a tube as
described in the Examples hereinbelow. The through pore or interstitial spaces

provide for the passage of eluting solvent and analyte materials. The separation is performed on the stationary surface. The surface can be porous, but is preferably nonporous. The form and function of the separations are identical to columns packed with beads. As with beads, the pores contained in the rod must be compatible with DNA and not trap the material. Also, the rod must not contain contamination that will trap DNA.

DEPR:
The molded polymeric rod of the present invention is prepared by bulk free radical polymerization within the confines of a chromatographic column. The base polymer of the rod can be produced from a variety of polymerizable monomers. For example, the **monolithic** rod can be made from polymers, including mono- and di-vinyl substituted aromatic compounds such as styrene, substituted styrenes, alpha-substituted styrenes and divinylbenzene; acrylates and methacrylates; polyolefins such as polypropylene and polyethylene; polyesters; polyurethanes; polyamides; polycarbonates; and substituted polymers including fluorosubstituted ethylenes commonly known under the trademark TEFLON. The base polymer can also be mixtures of polymers, non-limiting examples of which include poly(glycidyl methacrylate-co-ethylene dimethacrylate), poly(styrene-divinylbenzene) and poly(ethylvinylbenzene-divinylbenzene). The rod can be unsubsituted or substituted with a substituent such as a hydrocarbon alkyl or an aryl group. The alkyl group optionally has 1 to 1,000,000 carbons [illegible] branched chain, and includes straight chained, [illegible] saturated, unsaturated moieties functional groups of various types including aldehyde, ketone, ester, ether, alkyl groups, and the like, and the aryl groups includes as monocyclic, bicyclic, and tricyclic aromatic hydrocarbon groups including phenyl, naphthyl, and the like. In a

preferred embodiment, the alkyl group has 1-24 carbons. In a more preferred
embodiment, the alkyl group has 1-8 carbons. The substitution can also contain
hydroxy, cyano, nitro groups, or the like which are considered to be non-polar,
reverse phase functional groups. Methods for hydrocarbon substitution are
conventional and well-known in the art and are not an aspect of this invention.
The preparation of polymeric **monoliths** is by conventional methods well known in
the art as described in the following references: Wang et al. (J Chromatog. A
699:230 (1994)), Petro et al. (Ana. Chem. 68:315 (1996)), and the following
U.S. Pat. Nos. 5,334,310; 5,453,185; 5,522,994 (to Frechet). **Monolith** or
rod columns are commercially available form Merck & Co (Darmstadt, Germany).

DEPR:
A chromatography tube in which the **monolith** polymeric separation medium is
prepared is made of stainless steel. The monomers, styrene (Sigma--Aldrich
Chemical Corp.) and divinylbenzene (Dow Chemical Corp.) are dried over
magnesium sulfate and distilled under vacuum.

DEPR:
Following polymerization, the rubber plugs are replaced by column end fittings
and the column is connected to an HPLC system. The HPLC instrument has a
low-pressure mixing quaternary gradient capability. A cartridge or guard
column containing an iminodiacetate multivalent cation capture resin is placed
in line between the column and the mobile phase source reservoir. The column
is then washed by flowing 100 mL of tetrahydrofuran (THF) at 1 mL/min through
the column to remove the dodecyl alcohol and toluene, thereby creating
through-pores in the otherwise solid polymer **monolith**.

DEPR:
The non-polar, organic polymer **monolith** column is washed by flowing

tetrahydrofuran through the column at a flow rate of 2 mL per minute for 10
minutes followed by flowing methanol through the column at 2 mL per minute for
10 minutes. The non-polar, organic polymer **monolith** column is washed further
by flowing a mixture containing 100 mL of tetrahydrofuran and 100 mL of
concentrated hydrochloric acid through the column at 10 mL per minute for
minutes. Following this acid treatment, the non-polar, organic polymer
monolith column is washed by flowing tetrahydrofuran/water (1:1) through the
column at 2 mL per minute until neutral (pH 7).

DEPR:
Any double bonds remaining on the surface of the **monolith** column prepared in
Example 9 are reacted with bromine as described in Example 7.

DEPC:
Preparation of a Non-Polar Organic Polymer **Monolith**
Chromatography Column

DEPC:
Bromination of Remaining Double Bonds on the Surface of Non-Polar Organic
Polymer **Monolith** Column

DEPC:
Nitration of a Non-Polar Organic Polymer **Monolith** Column

CCXP:
210/198.2

ORPL:
Nakanishi et al. Double Pore Silica Gel **Monolith** Applied to Liquid
Chromatography, J. Sol-Gel Science & Technology, vol. 8, pp. 547-552, 1997.

ORPL:
Peters et al, Mixed **Monolithid** Rod of Macrophrous Poly(Sytrene-Co-Divinylbenzene) as a Separation Medium for FHLC of Syntntic
Polymer . . . , Analytical Chemistry, 68: 315-321 (1996).

DOCUMENT-IDENTIFIER: US 6296771 B1
TITLE: Parallel high-performance liquid chromatography with serial injection

BSPR:
Gel permeation chromatography (GPC), a well-known form of size exclusion
chromatography (SEC), is a frequently-employed chromatographic technique for
separation of samples generally, and for polymer size determination particular.
Another chromatographic separation approach is illustrated by U.S. Pat. No.
5,334,310 to Frechet et al. and involves the use of a porous **monolithic**
stationary-phase as a separation medium within the chromatographic column,
combined with a mobile-phase composition gradient. Other separation approaches
are also known in the art, including for example, normal-phase (e.g.,
adsorption) chromatography and reverse-phase chromatography, hydrophobic
interaction chromatography, hydrophilic interaction chromatography,
ion-exchange chromatography, affinity chromatography, among others.

DEPR:
The chromatographic column 102 (or a series of columns in one or more of the
chromatographic channels) further comprises a separation medium having a
stationary-phase within the separation cavity. The separation medium can
consist essentially of a stationary-phase or can also include, in addition
thereto, an inert support for the stationary phase. The column 102 can also
comprise frits, filters, fritted separation medium retention and/or for
filtering), and various fittings and features appropriate for preparing and/or
maintaining the column for its intended application. The particular separation
medium to be employed as the stationary-phase is not critical, and will

typically depend on the separation strategy for the particular chemistry of the polymer samples of interest, as well as on the desired detection, sample-throughput and/or information quality. Typical stationary-phase media can be a bed of packed beads, fibers, irregular or other shaped-particles, or a **monolithic** medium (typically greater than about 5 mm in thickness), each of which can be characterized and optimized for a particular separation strategy with respect to the material, size, shape, pore size, pore size distribution, surface area, solvent regain, bed homogeneity (for packed shaped-particles), inertness, polarity, hydrophobicity, chemical stability, mechanical stability and solvent permeability, among other factors. Generally preferred stationary-phase include porous media (e.g., porous beads, porous **monoliths**), such as are suitable for gel permeation chromatography (GPC), precipitation-redissolution chromatography, normal-phase (e.g., adsorption) chromatography and reverse-phase chromatography, hydrophobic interaction chromatography, hydrophilic interaction chromatography, ion-exchange chromatography, affinity chromatography, among others. Non-porous particles or empty columns and/or capillaries with adsorptive walls can be used as well. If beads are employed, spherical beads are preferred over other shapes. Particularly preferred stationary-phase media for polymer characterization applications are disclosed in greater detail below, but can generally include silica, cross-linked polymeric resins (e.g., poly(2-3-dihydroxypropylmethacrylate), poly(hydroxyethyl methacrylate), and polystyrenic polymers such as poly(styrene-divinylbenzene)).

CCVS:

210/198.2

DOCUMENT-IDENTIFIER: US 6290909 B1
TITLE: Sample injector for high pressure liquid chromatography

DEPR:
A sample injector A is also connected to common junction 335 between hydrostatic pressure source 310 and HPLC column 320. The injector is comprised of at least two elements such as 340 and 350. Each element comprises a container having an inlet and outlet end and filled with a dielectric material to form a porous bed within the container. Containers can include any geometric configuration capable of containing the porous bed of dielectric material, such as capillary tubes, and capable of withstanding pressures of up to about 40,000 psi. Also included are microchannels fabricated on a substrate such as those described by Paul et al. in U.S. Pat. Nos. 6,013,164 and 6,019,962 and by Arnold in prior co-pending U.S. patent application Ser. No. 09/404,945, filed Sep. 9, 1999, now U.S. Pat. No. 6,210,986, entitled "Microfluidic Channel Fabrication Method" assigned to the same assignee.
Elements 340 and 350 are connected together in series configuration with a common junction 335. The dielectric material filling each container is selected so as to minimize any chromatographic separation of the sample and can be any non-porous material, known to those skilled in the art, used to form a porous, packed bed. By way of example, the dielectric material can be comprised of granulated and nonporous silica, glass, or polymer beads or a porous monolithic polymer material. Further, the dielectric material is selected to resist pressure-driven flow but to allow electroosmotically-driven flow. Thus, additionally it is preferred that the pore diameter of the porous bed be in the range of about 25 to 300 nm. One of elements 340/350 serves as a

sample inlet
and is in communication with a sample container 360 and the other is connected
to a waste reservoir 370. A power supply 375 is connected across both elements
of sample injector A. Placing one of the electrodes of power supply 375 in the
sample container and the other in the waste reservoir can make this connection.
Alternatively, power supply 375 can be connected to the elements of the sample
injector by means of salt bridges, as discussed above.

CCXR:
210/198.2

DOCUMENT-IDENTIFIER: US 6287822 B1
TITLE: Mutation detection method

DEPR:
MIPC uses unique non-polar separation media which comprises organic polymers,
silica media having a non-polar surface comprising coated or covalently bound
organic polymers or covalently bound alkyl and/or aryl groups, continuous
non-polar separation media, so called **monolith** or rod columns, comprising
non-polar silica gel and organic polymer. The separation media used in MIPC
can be porous or non-porous. A detailed description of the MIPC separation
process, MIPC separation media, and MIPC systems is found in U.S. Pat. No.
5,772,889 (1998) to Gjerde and in co-pending U.S. patent application Ser. No.
09/058,560 filed Apr. 10, 1998; Ser. No. 09/058,337 filed Apr. 10, 1998 now
abandoned; Ser. No. 09/065,913 filed Apr. 24, 1998; now U.S. Pat. No.
5,986,095 Ser. No. 09/081,040 filed May 18, 1998; now U.S. Pat. No.
5,997,742 Ser. No. 09/081,039 filed May 18, 1998; now U.S. Pat. No.
5,972,222 and Ser. No. 09/080,547 filed May 18, 1998 now U.S. Pat. No.
6,017,457. MIPC systems and separation media are commercially available
(Transgenomic, Inc. San Jose, Calif.)

CCXR:
210/198.2

OREL:
Petro et al., Molded **Monolithid** Rod of Macroporous Polystyrene - [illegible] as a Separation Medium for HPLC of Synthtic Polymers . . . , Analytical Chemistry, 67:413-421 (1995).

DOCUMENT-IDENTIFIER: US 6260407 B1
TITLE: High-temperature characterization of polymers

BSPP:
Liquid chromatography is well known in the art for characterizing a polymer sample. Liquid chromatographic techniques employ separation of one or more components of a polymer sample from other components thereof by flow through a chromatographic column, followed by detection of the separated components with a flow-through detector. Approaches for liquid chromatography can vary, however, with respect to the basis of separation and with respect to the basis of detection. Gel permeation chromatography (GPC), a well-known form of size exclusion chromatography (SEC), is a frequently-employed chromatographic technique for polymer size determination. In GPC, the polymer sample is separated into components according to the hydrodynamic volume occupied by each component in solution. More specifically, a polymer sample is injected into a mobile phase of a liquid chromatography system and is passed through one or more chromatographic columns packed with porous beads. Molecules with relatively small hydrodynamic volumes diffuse into the pores of the beads and remain therein for longer periods, and therefore exit the column after molecules with relatively larger hydrodynamic volume. Hence, GPC can characterize one or more separated components of the polymer sample with respect to its effective hydrodynamic radius (Rh). Another [illegible] separation approach is illustrated by U.S. Pat. No. [illegible], to Frechet et al. and involves the use of a porous **monolithic** stationary-phase as a separation medium within the chromatographic column, combined with a mobile-phase composition gradient. (See also, Petro et al,

Molded **Monolithic**
Rod of Macroporous Poly(styrene-co-divinylbenzene) as a
Separation Medium for
HPLC Synthetic Polymers: "On-Column" Precipitation-Redissolution
Chromatography
as an Alternative to Size Exclusion Chromatography of Styrene
Oligomers and
Polymers, Anal. Chem., 68, 315-321 (1996); and Petro et al,
Immobilization of
Trypsin onto "Molded" Macroporous Poly (Glycidyl
Methacrylate-co-Ethylene
Dimethacrylate) Rods and Use of the Conjugates as Bioreactors and
for Affinity
Chromatography, Biotechnology and Bioengineering, Vol. 49, pp.
355-363
(1996)). Chromatography involving the porous **monolith** is
reportedly based on a
precipitation/redissolution phenomenon that separates the polymer
according to
size--with the precipitated polymer molecules selectively
redissolving as the
solvent composition is varied. The **monolith** provides the surface
area and
permeation properties needed for proper separation. Other
separation
approaches are also known in the art, including for example,
normal-phase
adsorption chromatography (with separation of polymer components
being based on
preferential adsorption between interactive functionalities of
repeating units
and an adsorbing stationary-phase) and reverse-phase
chromatography (with
separation of polymer components being based on hydrophobic
interactions
between a polymer and a non-polar stationary-phase). After
separation, a
detector can measure a property of the polymer or of a polymer
component--from
which one or more characterizing properties, such as molecular
weight, can be
determined as a function of time. Specifically, a number of
molecular-weight
distribution parameters can be determined, including for example: the
weight-average
molecular weight (M.sub.w), the number-average molecular weight
(M.sub.n), the
molecular-weight distribution shape, and an index of the breadth of
the
molecular-weight distribution (M.sub.w /M.sub.n), known as the

polydispersity
index (PDI). Other characterizing properties, such as mass, particle size,
composition or conversion can likewise be determined.

BSPR:
Aspects of polymer characterization, such as sample preparation and polymer
separation, have been individually and separately investigated. For example,
Poche et al. report a system and approach for automated high-temperature
dissolution of polymer samples. See Poche et al., Use of Laboratory Robotics
for Gel Permeation Chromatography Sample Preparation: Automation of
High-Temperature Polymer Dissolution, J. Appl. Polym. Sci., 64(8), 1613-1623
(1997). Stationary-phase media that reduce chromatographic separation times of
individual polymer samples have also been reported. See, for example, Petro et
al., Molded continuous poly(styrene-co-divinylbenzene) rod as a separation
medium for the very fast separation of polymers; Comparison of the
chromatographic properties of the **monolithic** rod with columns packed with
porous and no-porous beads in high-performance liquid chromatography., Journal
of Chromatography A, 752, 59-66 (1996); and Petro et al., Monodisperse
Hydrolyzed Poly(glycidyl methacrylate-co-ethylene dimethacrylate) Beads as a
Stationary Phase for Normal-Phase HPLC, Anal. Chem., 69, 3131 (1997). However,
such approaches have not contemplated nor been incorporated into protocols and
systems suitable for large-scale, or even moderate-scale, combinatorial
chemistry research, and particularly, for combinatorial material science
research methods. See, for example, U.S. Pat. No. [illegible] of polymers.

DETR:
The chromatographic column 102 further comprises a separation medium having a
stationary-phase within the separation cavity. The separation medium can
consist essentially of a stationary-phase or can also include, in

addition
thereto, an inert support for the stationary phase. The column 102 can also
comprise one or more fillers, frits (for separation medium retention and/or for
filtering), and various fittings and features appropriate for preparing and/or
maintaining the column for its intended application. The particular separation
medium to be employed as the stationary-phase is not critical, and will
typically depend on the separation strategy for the particular chemistry of the
polymer samples of interest, as well as on the desired detection, sample-throughput and/or information quality. Typical stationary-phase media
can be a bed of packed beads, rods or other shaped-particles, or a **monolithic**
medium (typically greater than about 5 mm in thickness), each of which can be
characterized and optimized for a particular separation strategy with respect
to the material, size, shape, pore size, pore size distribution, surface area,
solvent regain, bed homogeneity (for packed shaped-particles), inertness,
polarity, hydrophobicity, chemical stability, mechanical stability and solvent
permeability, among other factors. Generally preferred stationary-phase
include porous media (e.g., porous beads, porous **monoliths**), such as are
suitable for gel permeation chromatography (GPC), and media suitable for
precipitation-redissolution chromatography, adsorption chromatography, and/or
reverse-phase chromatography. Non-porous particles or empty columns and/or
capillaries with adsorptive walls can be used as well. If beads are employed,
spherical beads are preferred, but other shapes. Particularly preferred
[illegible] characterization applications are disclosed
in greater detail below, but can generally include silica, cross-linked resins,
hydroxylated polyglycidyl methacrylates, (e.g., poly(2-3-dihydroxypropylmethacrylate)), poly(hydroxyethyl methacrylate), and
polystyrenic polymers such as poly(styrene-divinylbenzene).

DEPR:
In other variations, the short column may comprise column stationary-phase packing other than is typically used for GPC, such as normal-phase or reverse-phase silica particles, polymer **monoliths,** inorganic **monoliths,** and other well-known column stationary-phase materials or filter media. For example, short columns containing adsorption chromatography stationary-phase can be used to remove components either more polar or less polar than the polymer sample of interest, such as water or solvents initially introduced with the sample. Also in a preferred aspect of this embodiment, more than one short column may be used in series, for example a short GPC column in combination with a short normal-phase adsorption chromatography column, such that polymer is separated from low-molecular-weight components, which are then further separated by polarity. (See Ex. 20). This can be particularly useful for rapidly separating polymer from residual monomer or solvent in a polymerization reaction, and then furtner quantifying the type and amount of monomer or solvent within a single, rapid analysis.

DEPR:
While some aspects of the following description refer to "beads", such reference is to be considered exemplary; other stationary-phase media (e.g., rods, **monoliths,** etc.) can be readily employed instead of such beads.

DEPR:
The interaction-replacement chromatography involves the use of mobile phase having a solvent gradient in conjunction with an insoluble stationary-phase (i.e., a polymer **monolith**). The polymer sample is injected into a mobile-phase solvent that is a "poor" solvent for the polymer being characterized (sometimes called a "non-solvent"), thereby causing precipitation of the

polymer sample.
The precipitated polymer sample then adsorbs onto the stationary-phase (e.g., **monolith**) surface. Gradually, a better solvent for the polymer being characterized is introduced into the mobile phase. When the better solvent contacts the precipitated polymer sample, the smaller particles of the polymer sample redissolve first. As more of the better solvent contacts the precipitated polymer sample, larger particles of the polymer sample redissolve, until the entire polymer sample has been redissolved. In this fashion, the polymer sample is separated by size (with the smaller particles corresponding to smaller size molecules). Solvent choices depend on the solubility characteristics of the polymer samples being characterized. For a typical hydrophobic polymer such as polystyrene, "good" solvents include tetrahydrofuran, toluene, dichloromethane, etc., while "poor" non-solvents include methanol, ethanol, water, or hexane. It is generally preferred that the good solvent and the poor solvent used for any particular separation be miscible.

DEPR:
The precipitation-redissolution chromatography approaches described herein--particularly employing **monolithic** columns such as those disclosed by Petro et al., vide supra., generally lead to high-speed characterization with good quality of information.

DEPR:
The stationary phase may be selected according to the type of polymer to be analyzed. Preferred stationary phases for this approach include porous **monoliths** and beads. Silica or hydrophilic polymer beads are used for adsorption of polar polymers or for removing of highly polar components of the samples, such as water, which would otherwise interfere with the analysis of

compounds of interest, such as monomers and polymers. Polymeric beads with diol functionalities are preferred for this purpose since they have higher adsorptivity than silica with minimized non-specific interactions with the characterized polymers (See M. Petro, et al., Anal. Chem., 1997, 69 3131; M. Petro, et al., J. Polym. Sci. A: Polym. Chem., 1997, 35, 1173; J. M. J. Frechet, et. al., Polym. Mater. Sci. Eng. 1997, 77, 38.).

DEPR:
The typical mobile phase (e.g., solvent) used for this adsorption chromatography is tetrahydrofuran, either alone or in mixtures with hexane (to enhance adsorption) or water (to enhance elution). Octadecyl-silica beads (commonly used in conventional reverse-phase HPLC) and polystyrene-based **monoliths** are used for a separation of compounds of medium polarity under the conditions typical of reversed-phase chromatography, usually in combination with a mixture of water and tetrahydrofuran. Optionally, gradients in connection with this technique can be employed, changing either the composition, temperature or flow rate of the mobile phase.

DEPR:
The robotic auto-sampler and injection valve set-up as in Example 1 was fitted with two sample loops (each having 50 microliter volume) in combination with a high-pressure liquid chromatographic (HPLC) apparatus comprising a two-pump gradient chromatography system, primed with methanol and tetrahydrofuran (THF) solvent. A porous crosslinked polystyrene **monolithic** column was utilized, prepared as described in Frechet et al., Journal of [illegible], 752, (1996) 59-66 and Frechet et al., Anal. Chem. 1996, 68, [illegible]. The HPLC system was configured such that the combined flow of the pump system passed through the valve, the column, and then to a UV chromatographic detector. The entire system, including pump control and data acquisition from

the detector
was computer-controlled.

DEPP:
Adsorption chromatography was used for separation of various components of the
reaction mixtures that contained the comonomers, (co)polymers, solvents and
catalyst components. Good separation was achieved in 60 seconds per sample
using a short, high-aspect ratio reversed-phase column and gradient of THF in
water with a concave profile. The specific gradient profile allows to separate
small molecules with similar retention behavior from each other as well as
elute a highly retained polymer in a very short time. Columns of various
sizes, porosities and chemistries were used for this purpose including
polystyrene-based **monoliths** and silica-based porous beads.

DEPR:
A single, short, high-aspect ratio column (0.6 cm.times.5 cm) contained a
polystyrene **monolith** as the separation medium and resided in a PL-210 HT-GPC
oven maintained at 140.degree. C. The system was configured substantially as
shown in FIG. 6 and described in connection therewith and as follows. Two
mobile-phase reservoirs 114, 120 were provided and equipped with two Waters 515
pumps 116, 118. A "mobile-phase A" reservoir 114 feeding pump 116 (hereinafter
"pump A") comprised trichlorobenzene (TCB) and, in operation, was configured to
pump mobile-phase A through the injection valve 210 (100) and through the oven,
wherein the mobile-phase A was heated to become the hot mobile phase (i.e., hot
TCB). A "mobile-phase B" reservoir 120 feeding pump 118 (hereinafter "pump B").
The comprised trichlorobenzene, and in operation, was configured to pump
mobile-phase B to bypass most of the heated environment, and to enter the oven
immediately prior to the column 102 as an essentially ambient-temperature
mobile phase (i.e., cold TCB). Detection was effected with a PD

2000
light-scattering detector (90.degree.).

CCXP:
210/198.2

ORPL:
Petro et al., 1996, I.J. Chromotography A, 752: 59-66 Molded continuous poly
(styrene-co-divinylbenzene) rod as a separation medium for the very fast
separation of polymers Comparison of the chromatographic properties of the
monolithic rod with columns packed with porous and non-porous beads in
high-performance liquid chromatography of polystyrenes.

ORPL:
Petro et al., Analytical Chemistry, 1996, vol. 68: 315-321 Molded **monolithic**
rod of macroporous Poly(styrene-co-divinylbenzene) as a Separation Medium for
HPLC of Synthetic Polymers: "on-Column" Precipitation-Redissolution
Chromatography as an Alternative to Size Exclusion Chromatography of Styrene
Oligomers and Polymers.

DOCUMENT-IDENTIFIER: US 6258264 B1
TITLE: Non-polar media for polynuclectide separations

BSPR:
The present invention is directed to the separation of polynuclectides using a separation medium having non-polar surfaces, such as the surfaces of nonporous beads or surfaces of interstitial spaces within a molded **monolith** (e.g., a derivatized silica **monolith**), which surfaces are substantially free from contamination with multivalent cations. More specifically, the invention is directed to the chromatographic separation of both single stranded and double stranded polynucleotides by chromatography using a nonporous separation medium, where the medium is either organic or inorganic material which is coated with a polymer, or non-polar substituted polymer, and/or which has substantially all surface substrate groups substituted with a non-polar hyydrocarbon or non-ionic substituted hydrocarbon.

BSPR:
These and other objects of the invention, which will become apparent from reading the following specification, have been achieved by the method of the present invention in which polynucleotides are separated using a nonporous separation medium such as beads or a molded **monolith** (e.g., a silica gel **monolith**), where the medium comprises either organic or inorganic material which is coated with a polymer, or non-polar substituted polymer, and/or which has substantially all surface substrate groups substituted with a non-polar hydrocarbon or non-ionic substituted hydrocarbon.

BSPR:
In one aspect, the invention is a method for separating a mixture of polynucleotides comprising applying a mixture of polynucleotides

having up to
1500 base pairs to a separation medium, the separation surfaces of the medium
coated with a hydrocarbon or non-polar hydrocarbon substituted polymer, or
having substantially all polar groups reacted with a non-polar hydrocarbon or
substituted hydrocarbon group, wherein said surfaces are non-polar; and eluting
the polynucleotides. The separation medium can be enclosed in a column.
Examples of non-polar surfaces include the surfaces of beads such as nonporous
particles and the surfaces of intersitital spaces within a monolith (e.g., a
silica gel monolith), which surfaces are coated with a hydrocarbon or non-polar
substituted polymer or having substantially all surface substrate groups
reacted with a non-polar hydrocarbon or substituted hydrocarbon group. In the
preferred embodiment, precautions are taken during the production of the medium
so that it is substantially free of multivalent cation contaminants and the
medium is treated, for example by an acid wash treatment and/or treatment with
multivalent cation binding agent, to substantially remove any residual surface
metal contaminants. The preferred separation medium is characterized by having
a DNA Separation Factor (defined hereinbelow) of at least 0.05. The preferred
medium is characterized by having a Mutation Separation Factor (as defined
hereinbelow) of at least 0.1. In a preferred embodiment, the separation is
made by Matched Ion Polynucleotide Chromatography (MIPC, as defined
hereinbelow). The elution step preferably uses a mobile phase containing a
counterion agent and a water-soluble organic solvent. Examples of a counterion
organic solvent include alcohol, nitrile, dimethylformamide, tetrahydrofuran,
ester, ether, and mixtures of one or more thereof, e.g., methanol, ethanol,
2-propanol, 1-propanol, tetrahydrofuran, ethyl acetate, acetonitrile. The most
preferred organic solvent is acetonitrile. The counterion agent

is preferably
selected from the group consisting of lower primary amine, lower secondary
amine, lower tertiary amine, lower trialkyammonium salt, quaternary ammonium
salt, and mixtures of one or more thereof. Non-limiting examples of counterion
agents include octylammonium acetate, octyidimethylammonium acetate,
decylammonium acetate, octadecylammonium acetate,
pyridiniumammonium acetate,
cyclohexylammonium acetate, diethylammonium acetate, propylethylammonium
acetate, propyidiethylammonium acetate, butylethylammonium acetate,
methylhexylammonium acetate, tetramethylammonium acetate, tetraethylammonium
acetate, tetrapropylammonium acetate, tetrabutylammonium acetate,
dimethydiethylammonium acetate, triethylammonium acetate, tripropylammonium
acetate, tributylammonium acetate, and mixtures of any one or more of the
above. The counterion agent includes an anion, e.g., acetate, carbonate,
bicarbonate, phosphate, sulfate, nitrate, propionate, formate, chloride,
perchlorate, or bromide. The most preferred counterion agent is
triethylammonium acetate or triethylammonium hexafluoroisopropyl alcohol.

BRIEF:
In a still further aspect, the invention is a method for separating a mixture
of polynucleotides comprising applying a mixture of polynucleotides having up
to 1500 base pairs to a **monolith** having non-polar separation surfaces, and
eluting the polynucleotides. The **monolith** can be enclosed in a column or other
containment system, such as a cartridge. In a preferred embodiment, the
monolith is a silica gel **monolith**. The non-polar separation surfaces are
the surfaces of intersitital spaces within the **monolith**, which surfaces are
coated with a hydrocarbon or non-polar substituted polymer or having
substantially all surface substrate groups reacted with a non-polar hydrocarbon
or substituted hydrocarbon group. An example of a suitable

monolith is one which is polyfunctionally derivatized with octadecylsilyl groups. In the preferred embodiment, precautions are taken during the production of the monolith so that it is substantially free of multivalent cation contaminants and the monolith is treated, for example by an acid wash treatment and/or treatment with multivalent cation binding agent, to substantially remove any residual surface metal contaminants. The preferred monolith is characterized by having a DNA Separation Factor of at least 0.05. The preferred monolith is characterized by having a Mutation Separation Factor of at least 0.1. In a preferred embodiment, the separation is made by Matched Ion Polynucleotide Chromatography. The elution step preferably uses a mobile phase containing a counterion agent and a water-soluble organic solvent. Examples of a suitable organic solvent include alcohol, nitrile, dimethylformamide, tetrahydrofuran, ester, ether, and mixtures of one or more thereof, e.g., methanol, ethanol, 2-propanol, 1-propanol, tetrahydrofuran, ethyl acetate, acetonitrile. The most preferred organic solvent is acetonitrile. The counterion agent is preferably selected from the group consisting of lower primary amine, lower secondary amine, lower tertiary amine, lower trialkyammonium salt, quaternary ammonium salt, and mixtures of one or more thereof. Non-limiting examples of counterion agents include octylammonium acetate, octyldimethylammonium acetate, [illegible]ylammonium acetate, octadecylammonium acetate, pyridiniumammonium acetate, [illegible]propylammonium acetate, diethylammonium acetate, propylethylammonium acetate, [illegible]pyldiethylammonium acetate, butylethylammonium acetate, [illegible]thylhexylammonium acetate, tetramethylammonium acetate, tetraethylammonium acetate, tetrapropylammonium acetate, tetrabutylammonium acetate, dimethydiethylammonium acetate, triethylammonium acetate, tripropylammonium

acetate, tributylammonium acetate, and mixtures of any one or more of the above. The counterion agent includes an anion, e.g., acetate, carbonate, bicarbonate, phosphate, sulfate, nitrate, propionate, formate, chloride, perchlorate, or bromide. The most preferred counterion agent is triethylammonium acetate or triethylammonium hexafluoroisopropyl alcohol.

BSPR:
In a yet further aspect, the invention provides a **monolith** having non-polar separation surfaces which are substantially free from contamination with multivalent cations. The **monolith** can be enclosed in a column or other containment system, such as a cartridge. The non-polar separation surfaces include the surfaces of interstitial spaces within the **monolith** (e.g., a silica **monolith**), which surfaces are coated with a hydrocarbon or non-polar substituted polymer or having substantially all surface substrate groups reacted with a non-polar hydrocarbon or substituted hydrocarbon group. An example of a suitable **monolith** is one which is derivatized with polyfunctionally derivatized octadecylsilyl groups. In the preferred embodiment, precautions are taken during the production of the **monolith** so that it is substantially free of multivalent cation contaminants and the **monolith** is treated, for example by an acid wash treatment and/or treatment with multivalent cation binding agent, to remove any residual surface metal contaminants. The preferred **monolith** is characterized by having a DNA separation factor of at least [illegible]. The preferred **monolith** is characterized by having a [illegible] of at least [illegible].

BSPR:
In another aspect, the present invention is a method for treating the non-polar surfaces of a medium used for separating polynucleotides, such as the surfaces of beads in a MIPC column or the surfaces of interstitial spaces

in a **monolith,**
in order to improve the resolution of polynucleotides, such as dsDNA, separated
on said surfaces. This treatment includes contacting the surface with a
solution containing a multivalent cation binding agent. In a preferred
embodiment, the solution has a temperature of about 50.degree. C. to
90.degree. C. An example of this treatment includes flowing a solution
containing a multivalent cation binding agent through a MIPC column, wherein
the solution has a temperature of about 50.degree. C. to 90.degree. C. The
preferred temperature is about 70.degree. C. to 80.degree. C. In a preferred
embodiment, the multivalent cation binding agent is a coordination compound,
examples of which include water-soluble chelating agents and crown ethers.
Specific examples include acetylacetone, alizarin, aluminon, chloranilic acid,
kojic acid, morin, rhodizonic acid, thionalide, thiourea, .alpha.-furildioxime,
nioxime, salicylaldoxime, dimethylglyoxime, .alpha.-furildioxime, cupferron,
.alpha.-nitroso-.beta.-naphthol, nitroso-R-salt, diphenylthiocarbazone,
diphenylcarbazone, eriochrome black T, PAN, SPADNS, glyoxal-bis(2-hydroxyanil),
murexide, .alpha.-benzoinoxime, mandelic acid, anthranilic acid,
ethylenediamine, glycine, triaminotriethylamine, thionalide,
triethylenetetramine, ethylenediaminetetraacetic acid (EDTA), metalphthalein,
arsonic acids, .alpha.,.alpha.'-bipyridine, 4-hydroxybenzothiazole,
8-hydroxyquinaldine, 8-hydroxyquinoline, 1,10-phenanthroline, picolinic acid,
quinaldic acid, .alpha.,.alpha.',.alpha."-terpyridyl, [illegible],
acid, tiron, [illegible], rubeanic acid, [illegible], and zinc
dibenzyldithiocarbamate. However, the most preferred chelating agent is EDTA.
In this aspect of the invention, the solution preferably includes an organic
solvent as exemplified by alcohol, nitrile, dimethylformamide,

tetrahydrofuran,
ester, ether, and mixtures thereof. Examples of suitable solvents include
methanol, ethanol, 2-propanol, 1-propanol, tetrahydrofuran, ethyl acetate,
acetonitrile, and mixtures thereof. The most preferred organic solvent is
acetonitrile. In one embodiment, the solution can include a counterion agent
such as lower primary, secondary and tertiary amines, and lower trialkyammonium
salts, or quaternary ammonium salts. More specifically, the counterion agent
can be octylammonium acetate, octadimethylammonium acetate, decylammonium
acetate, octadecylammonium acetate, pyridiniumammonium acetate, cyclohexylammonium acetate, diethylammonium acetate, propylethylammonium
acetate, propyldiethylammonium acetate, butylethylammonium acetate,
methylhexylammonium acetate, tetramethylammonium acetate, tetraethylammonium
acetate, tetrapropylammonium acetate, tetrabutylammonium acetate, dimethydiethylammonium acetate, triethylammonium acetate, tripropylammonium
acetate, tributylammonium acetate, tetraethylammonium acetate, tetrapropylammonium acetate, tetrabutylammonium acetate, and mixtures of any
one or more of the above. The counterion agent includes an anion, e.g.,
acetate, carbonate, bicarbonate, phosphate, sulfate, nitrate, propionate,
formate, chloride, perchlorate, and bromide. However, the most preferred
counterion agent is triethylammonium acetate.

BSPR:
In yet a further aspect, the invention provides a method for storing a medium
used for separating polynucleotides, e.g., the beads of a MIPC monolith, in order to improve the resolution of double stranded polynucleotides
separated using the medium. In the case of a MIPC column, the preferred method
includes flowing a solution containing a multivalent cation binding agent
through the column prior to storing the column. In a preferred embodiment, the
multivalent cation binding agent is a coordination compound,

examples of which
include water-soluble chelating agents and crown ethers.
Specific examples
include acetylacetone, alizarin, aluminon, chloranilic acid,
kojic acid, morin,
rhodizonic acid, thionalide, thiourea, .alpha.-furildioxime,
nioxime,
salicylaldoxime, dimethylglyoxime, .alpha.-furildioxime,
cupferron,
.alpha.-nitroso-.alpha.-naphthol, nitroso-R-salt,
diphenylthiocarbazone,
diphenylcarbazone, eriochrome black T, PAN, SPADNS,
glyoxal-bis(2-hydroxyanil),
murexide, .alpha.-benzoinoxime, mandelic acid, anthranilic acid,
ethylenediamine, glycine, triaminotriethylamine, thionalide,
triethylenetetramine, EDTA, metalphthalein, arsonic acids,
.alpha.,.alpha.'-bipyridine, 4-hydroxybenzothiazole,
8-hydroxyquinaldine,
8-hydroxyquinoline, 1,10-phenanthroline, picolinic acid,
quinaldic acid,
.alpha.,.alpha.',.alpha."-terpyridyl,
9-methyl-2,3,7-trihydroxy-6-fluorone,
pyrocatechol, salicylic acid, tiron,
4-chloro-1,2-dimercaptobenzene, dithiol,
mercaptobenzothiazole, rubeanic acid, oxalic acid, sodium
diethyldithiocarbarbamate, and zinc dibenzyldithiocarbamate.
However, the most
preferred chelating agent is EDTA. In this aspect of the
invention, the
solution preferably includes an organic solvent as exemplified by
alcohols,
nitriles, dimethylformamide, tetrahydrofuran, esters, and ethers.
The most
preferred organic solvent is acetonitrile. The solution can also
include a
counterion agent such as lower primary, secondary and tertiary
amines, and
lower trialkyammonium salts, or quaternary ammonium salts. More
specifically,
the counterion agent can be octylammonium acetate,
[illegible]
acetate, hexylammonium acetate, octadecylammonium acetate,
[illegible]
acetate, cyclohexylammonium acetate, diethylammonium acetate,
propylethylammonium acetate, propyldiethylammonium acetate,
butylethylammonium
acetate, methylhexylammonium acetate, tetramethylammonium
acetate,
tetraethylammonium acetate, tetrapropylammonium acetate,
tetrabutylammonium
acetate, dimethydiethylammonium acetate, triethylammonium

acetate,
tripropylammonium acetate, tributylammonium acetate,
tetraethylammonium
acetate, tetrapropylammonium acetate, tetrabutylammonium acetate,
and mixtures
of any one or more of the above. The counterion agent includes
an anion, e.g.,
acetate, carbonate, bicarbonate, phosphate, sulfate, nitrate,
propionate,
formate, chloride, perchlorate, and bromide. However, the most
preferred
counterion agent is triethylammonium acetate.

DEPR:
The medium can be enclosed in a column. In one embodiment, the
non-polar
surfaces comprise the surfaces of beads. In an alternative
embodiment, the
surfaces comprise the surfaces of interstitial spaces in a molded
monolith.
For purposes of simplifying the description of the invention and
not by way of
limitation, the separation of polynucleotides using nonporous
beads, and the
preparation of such beads, will be primarily described herein, it
being
understood that other separation surfaces, such as the
interstitial surfaces of
monoliths, are intended to be included within the scope of this
invention.
Monoliths such as derivatized silica gel rods contain separation
media which
have been formed inside a column as a unitary structure having
through pores or
interstitial spaces which allow eluting solvent and analyte to
pass through and
which provide the non-polar separation surface.

DEPR:
In another embodiment of the present invention, the separation
media can be in
the form of a monolith such as a rod-like monolithic column. The
monolithic
column can be polymerized or formed as a single unit inside of a
tube. The
through pore or interstitial spaces provide for the passage of
eluting solvent
and analyte materials. The separation is performed on the
stationary surface.
The surface can be porous, but is preferably nonporous. The form

and function
of the separations are identical to columns packed with beads. As with beads,
the pores contained in the rod must be compatible with DNA and not trap the
material. Also, the rod must not contain contamination that will trap DNA.

DEPR:
In one embodiment of the present invention, the separation medium is continuous
monolithic silica gel. A molded **monolith** can be prepared by polymerization
within the confines of a chromatographic column (e.g., to form a rod) or other
containment system. A **monolith** is preferably obtained by the hydrolysis and
polycondensation of alkoxysilanes. A preferred **monolith** is derivatized in
order to produce non-polar interstitial surfaces. Chemical modification of
silica **monoliths** with octadecyl, methyl or other ligands can be carried out.
An example of a preferred derivatized **monolith** is one which is polyfunctionally
derivatized with octadecylsilyl groups. The preparation of derivatized silica
monoliths is by conventional methods well known in the art as described in
Example 15 and in the following references which are hereby incorporated in
their entirety herein: Nakanishi, et al., J. Sol-Gel Sci. Technol. 8:547
(1997); Nakanishi, et al., Bull, Chem. Soc. Jpn. 67:1327 (1994); Cabrera, et
al., Trends Analytical Chem. 17:50 (1998); Jinno, et al., Chromatographia
27:199 (1989).

DEPR:
[illegible] non-polar, derivatized silica **monolith** column is washed by flowing
[illegible] minute for 10
minutes followed by flowing methanol through the column at 4 mL per minute for
15 minutes. The non-polar **monolith** column is washed further by flowing a
mixture containing 100 mL of tetrahydrofuran and 100 mL of concentrated

hydrochloric acid through the column at 10 mL per minute for 20 minutes.
Following this acid treatment, the **monolith** column is washed by flowing
tetrahydrofuran/water (1:1) through the column at 2 mL per minute until neutral
(pH 7).

DEPC:
Preparation of a Silica **Monolith**

CCOR:
210/198.2

OBPL:
Nakanishi et al. Double Pore Silica Gel **Monolith** Applied to Liquid
Chromatography, J. Sol-Gel Science & Technology, vol. 8, pp. 547-552, 1997.

OBPL:
Petro et al, Molded **Monolithid** Rod of Macrophrous
Poly(Styrene-CO-Divinylbenzene) as a Separation Medium for PHLC of Synthtic
Polymers . . . , Analytical Chemistry, 68: 315-321 (1996).

DOCUMENT-IDENTIFIER: US 6245227 B1
TITLE: Integrated **monolithic** microfabricated electrospray and liquid
chromatography system and method

TTL:
Integrated **monolithic** microfabricated electrospray and liquid chromatography
system and method

ABPL:
An electrospray device, a liquid chromatography device and an electrospray-liquid chromatography system are disclosed. The electrospray
device comprises a substrate defining a channel between an entrance orifice on
an injection surface and an exit orifice on an ejection surface, a nozzle
defined by a portion recessed from the ejection surface surrounding the exit
orifice, and an electrode for application of an electric potential to the
substrate to optimize and generate an electrospray; and, optionally, additional
electrode(s) to further modify the electrospray. The liquid chromatography
device comprises a separation substrate defining an introduction channel
between an entrance orifice and a reservoir and a separation channel between
the reservoir and an exit orifice, the separation channel being populated with
separation posts perpendicular to the fluid flow; a cover substrate bonded to
the separation substrate to enclose the reservoir and the separation channel
adjacent the cover substrate; and, optionally, electrode(s) for application of
a electric potential to the fluid. The exit orifice of the liquid
chromatography device may be homogeneously interfaced with the entrance orifice
of the electrospray device to form an integrated single system. An array of
multiple systems may be fabricated in a single **monolithic** chip for rapid
sequential fluid processing and generation of electrospray for

subsequent
analysis, such as by positioning the exit orifices of the electrospray devices
near the sampling orifice of a mass spectrometer.

PCPR:
This application is related to copending U.S. application Ser. No.
09/156,507, entitled INTEGRATED **MONOLITHIC** MICROFABRICATED ELECTROSPRAY AND
LIQUID CHROMATOGRAPHY SYSTEM AND METHOD, filed Sep. 17, 1998.

BSPR:
The present invention relates generally to an integrated miniaturized chemical
analysis system fabricated using microelectromechanical systems (MEMS)
technology. In particular, the present invention relates to an integrated
monolithic microfabricated electrospray and liquid chromatography device. This
achieves a significant advantage in terms of high-throughput analysis by mass
spectrometry, as used, for example, in drug discovery, in comparison to a
conventional system.

BSPR:
In all of the above-described devices, edge-spraying from a **monolithic** chip is
a poorly controlled process due to the inability to rigorously and repeatably
determine the physical form of the chip's edge. In another embodiment of
edge-spraying, ejection nozzles, such as small segments of drawn capillaries,
are separately and individually attached to the chip's edge. This process is
inherently cost-inefficient and unreliable, imposes space constraints in chip
design, and is thereby undesirable for manufacturing.

DSPR:
The fabrication of the electrospray device 100 will now be explained with
reference to FIGS. 9A-20B. The electrospray device 100 is preferably
fabricated as a **monolithic** silicon integrated circuit utilizing established,
well-controlled thin-film silicon processing techniques such as

thermal
oxidation, photolithography, reactive-ion etching (RIE), ion implantation, and
metal deposition. Fabrication using such silicon processing techniques
facilitates massively parallel processing of similar devices, is time- and
cost-efficient, allows for tighter control of critical dimensions, is easily
reproducible, and results in a wholly integral device, thereby eliminating any
assembly requirements. Further, the fabrication sequence may be easily
extended to create physical aspects or features on the injection surface and/or
ejection surface of the electrospray device to facilitate interfacing and
connection to a fluid delivery system or to facilitate integration with a fluid
delivery sub-system to create a single integrated system.

DEPR:
The above described fabrication sequence for the electrospray device 100 can be
easily adapted to and is applicable for the simultaneous fabrication of a
single **monolithic** system comprising multiple electrospray devices including
multiple channels and/or multiple ejection nozzles embodied in a single
monolithic substrate. Further, the processing steps may be modified to
fabricate similar or different electrospray devices merely by, for example,
modifying the layout design and/or by changing the polarity of the photomask
and utilizing negative-working photoresist rather than utilizing
positive-working photoresist.

DEPR:
The upstream fluid delivery device 318 may be a **monolithic** integrated circuit
[illegible] through which a fluid sample can pass directly or
indirectly to the entrance orifice of the electrospray device 100. The
upstream fluid delivery device 318 may be a silicon microchip-based liquid
separation device capable of, for example, capillary electrophoresis, capillary

electrochromatography, affinity chromatography, liquid chromatography (LC) or
any other condensed-phase separation methods. Further, the upstream fluid
delivery device 318 may be a silicon, glass, plastic and/or polymer based
device such that the electrospray device 100 may be chip-to-chip or
wafer-to-wafer bonded thereto by any suitable method. An example of a
monolithic liquid chromatography device for utilization in, for example, the
single integrated system 316, is described below.

DEPR:
The silicon-based liquid chromatography device 400 reduces the size of a
typical liquid chromatography device by nearly two orders of magnitude. The
dimensional scaling may provide the advantage of significantly reducing the
mass of the analyte and/or the volume of the fluid sample required for accurate
analysis. Further, by reducing a macroscopic separation column and its packing
materials to a **monolithic** device, the liquid chromatography device 400 can be a
component of an on-chip integrated system.

DEPR:
Referring now to FIGS. 30-35, although the liquid chromatography device has
been described as comprising a single reservoir and a single separation
channel, the **monolithic** liquid chromatography device may be easily adapted and
modified to comprise multiples of the liquid chromatography device and/or
multiple entrance orifices, exit orifices, reservoirs and/or separation
[illegible] In each of the variations, any or all of the reservoir(s),
[illegible], and separation roots may have different dimensions
and/or shapes.

DEPR:
The fabrication of the liquid chromatography device of the present invention
will now be explained with reference to FIGS. 36A-46C. The

liquid
chromatography device is preferably fabricated as a **monolithic** silicon micro
device utilizing established, well-controlled thin-film silicon processing
techniques such as thermal oxidation, photolithography, reactive-ion etching
(RIE), ion implantation, and metal deposition. Fabrication using such silicon
processing techniques facilitates massively parallel processing of similar
devices, is time- and cost-efficient, allows for tighter control of critical
dimensions, is easily reproducible, and results in a wholly integral device,
thereby eliminating any assembly requirements. Manipulation of separate
components and/or sub-assemblies to build an liquid chromatography device with
high reliability and yield is not desirable and may not be possible at the
micrometer dimensions required for efficient separation.

DEPR:
The above described fabrication sequence for the liquid chromatography device
may be easily adapted to and is applicable for the simultaneous fabrication of
a **monolithic** system comprising multiple liquid chromatography devices including
multiple reservoirs and/or multiple separation channels as described above
embodied in a single **monolithic** substrate.

CCOR:
210/198.2

DOCUMENT-IDENTIFIER: US 6238565 B1
TITLE: **Monolithic** matrix for separating bio-organic molecules

TTL:
Monolithic matrix for separating bio-organic molecules

ABPL:
The present invention provides **monolithic** polymer matrices for the separation of bio-organic molecules by liquid chromatography. In one embodiment, the matrix is formed from a polymerization mixture including (i) a hydrophobic monomer, (ii) a crosslinking agent, and (iii) a porogenic solvent or mixture of porogenic components. The **monolithic** matrices of the invention are particularly useful for resolving polynucleotides (e.g., DNA and/or RNA) in samples by way of reversed-phase ion-pairing chromatography.

BSPR:
The present invention relates to the separation of bio-organic molecules. In particular, the invention provides a **monolithic** polymer matrix for the separation of polynucleotides by reversed-phase ion-pairing chromatography.

BSPR:
The above theory relating to beds of packed particles is not particularly useful for predicting the behavior of macromolecules in continuous **monolithic** beds, where mass transport may be a combination of diffusive and convective processes.

BSPR:
The present invention, [illegible] **monolithic** [illegible] separating mixtures containing polynucleotides, is based in part on the discovery that **monoliths** provide reduced pressure drops corresponding to the use of largeparticle stationary phases while maintaining the separation resolution of columns packed

with small spherical particles. More particularly, it has been discovered that reversed-phase **monolithic** matrices can provide an improved method for the high speed separation of DNA molecules and that such separations can be performed with high resolution at greatly reduced operating pressures compared to previously available methods. This surprising finding now permits the high-resolution separation of polynucleotides under conditions not possible with preexisting technology. Moreover, the **monolithic** columns of the present invention can be constructed with stationary phase geometries significantly different than those available with packed beds. The effects of such novel geometries on the separation of macromolecules have not been predicted so far by current chromatographic theory.

BSPR:
The **monolithic** columns of the present invention provide all of the advantages of the previous best technology for polynucleotide separations (i.e., packed beds of alkylated nonporous polymer beads), without the need to tediously prepare beads and pack them into efficient columns. The columns produced by the current invention are easily prepared using simple processes and once prepared, cannot fail through shifting within a packed bed because there are no individual beads to shift position.

BSPR:
In addition to the improved ease of manufacturing of the new columns and lack of bead shifting, the **monolithic** columns [illegible] provide [illegible] at least 50% better resolution than that expected for a column of packed spheres when normalized for operating pressures.

BSPR:
One aspect of the present invention provides a method for

resolving a mixture containing at least one polynucleotide (e.g., DNA and/or RNA). According to the method, the mixture is passed through a **monolithic** polymer matrix held in a stationary fashion by a support. The mixture is separated by ion-pair reverse-phase chromatography.

BSPP:
In one embodiment, the **monolithic** polymer matrix has hydrophobic surface groups. The **monolithic** polymer matrix can be comprised, at least in part, of a polymer selected from the group consisting of polymethacrylates and polystyrenes. In one embodiment, the polymer is a polymethacrylate.

PSPP:
According to one embodiment, the **monolithic** polymer matrix is formed from a polymerization mixture induding a hydrophobic monomer, a crosslinking agent, and a porogen. The porogen may be (i) a porogenic solvent, (ii) a mixture of porogenic solvents, or (iii) one or more porogenic solvents containing at least one polymeric additive that contributes to pore formation. The hydrophobic monomer can be an alkyl methacrylate.

BSPP:
In one embodiment, the method further includes the step of passing an eluant containing an ion-pairing agent through the **monolithic** matrix. The separation is carried out under the driving force of a reasonable pressure (e.g., less than about 5,000 psi).

BSPP:
In additional embodiments, the [illegible] support. The **monolithic** polymer matrix may fill a channel formed in the plate, or it may take the form of a thin film on the plate.

BSPP:
Another aspect of the invention provides a chromatographic

apparatus comprising
a support holding a macroporous **monolithic** methacrylate-based polymer matrix.
In one embodiment, the polymer matrix has a hydrophobic surface capable of
interacting with hydrophobic groups of an ion-pairing agent for resolving
polynucleotides by reverse-phase ion-pair chromatography. The polymer matrix
may have a relatively high void fraction (e.g., greater than about 0.6).

ESPR:
In another embodiment, the kit includes: (i) a **monolithic** polymer matrix having
hydrophobic surface groups held in a stationary fashion by a support; and (ii)
an ion-pairing agent capable of interacting with negatively charged phosphate
groups of the polynucleotides and also with the hydrophobic surface groups of
the monomer.

DRFR:
FIG. 1 is a chromatogram showing the separation of oligothymidylic acids
between 12 to 18 units in length on a C6 **monolithic** column constructed in
accordance with an embodiment of the present invention.

DRFR:
FIG. 2A is a chromatogram showing the separation of double-stranded DNA
fragments on a C12 **monolithic** column constructed in accordance with an
embodiment of the present invention.

DRFR:
FIG. 3A is a chromatogram showing the separation of homoduplex DNA fragments
[illegible] **monolithic** C12 column
[illegible]

DRFR:
FIG. 3B is a chromatogram showing the separation of a mixture containing the
same type of homoduplex DNA fragments of FIG. 3A as well as a variant sequence
containing a single base pair substitution on a porous **monolithic**

C12 column
constructed in accordance with an embodiment of the present invention.

DEPR:
The present invention provides a **monolithic** bed, and method of making and using
the same, for resolving bio-organic molecules.

DEPR:
Any suitable crosslinking agents known to those skilled in art may be employed
in forming the **monolithic** matrix of the invention. Preferred crosslinking
monomers contain at least two carbon-carbon double bonds capable of
polymerization in the presence of an initiator. Exemplary crosslinking
monomers include divinyl benzene, butadiene, trimethylolpropane trimethacrylate
(TRIM), etc.

DEPR:
For TRIM-based polymerization mixtures, highly permeable **monoliths** will result,
for example, from the use of pure Isooctane as a porogenic solvent. Lower
permeability will result, for example, from addition of 5-20% of other
solvents, such as 2-octanone, toluene, and/or ethyl proprionate, to the
isooctane. Permeability may also be reduced by increasing the ratio of
hydrophobic monomer to crosslinker, and by decreasing the proportion of
porogenic solvent in the mixture. Additionally, the choice of initiator may be
used as a means to control the pore distribution.

DEPR:
In an exemplary embodiment, the **monolithic** matrix is prepared from a
polymerizable mixture of trimethylolpropane trimethacrylate, an alkyl methacrylate, an alkane porogenic
solvent or mixture of porogenic components, and a free radical initiator. For
example, a polymerization mixture was prepared using trimethylolpropane
trimethacrylate, an alkyl methacrylate, isooctane, and azobisisobutyronitrile.

The mixture was poured into an empty HPLC column tube, capped, and polymerized
at 65-70.degree. C. for 8-24 hours. The polymerized matrix was then equipped
with endfittings and flushed with solvent to remove unreacted components.

DEPR:
A typical **monolithic** column constructed in accordance with the present
invention will have pores in the less than 5,000 nm range, down to about 10 nm.
The size of larger pores can be determined using very large DNA and/or
microscopic exam. For example, the columns of the invention have been used to
separate DNA products of 2,072, 2,647, and 3,147 base pairs in length. The
largest of these would correspond to a molecular size of at lease 1 micron.
Accordingly, the **monolithic** column used to separate this DNA would be expected
to include pores having a diameter of at least 1 micron. This is in agreement
with permeability calculations that indicate an apparent particle diameter of 6
microns, or a pore size of about 2 microns. Microscopic exam revealed that a
typical matrix of the invention includes small globules of about 1-2 microns in
diameter that are fused into continuous structures with pores in the 1-3 micron
size range. This is consistent with the estimated pore size based on
permeability and DNA fragment chromatography.

DEPR:
Once prepared, the **monolithic** matrix of the Invention Is useful for resolving
bio-organic molecules, for example, by gradient or isocratic liquid
chromatography. In an exemplary use, a column of the invention, prepared as
described above, is employed to carry out a separation of polynucleotides
contained in a sample. In one embodiment, water and acetonitrile, both
containing an ion-pairing agent, are used in gradient liquid chromatography to
elute polynucleotides injected into the column.

DEPR:
A variety of support structures may be used with the **monolithic** matrix of the invention. For example, one embodiment contemplates the use of an elongated column tube. In another embodiment, the **monolithic** matrix is held in the lumen of a capillary tube. In these embodiments, the matrix extends across the entire cross-sectional area of the tube. The tube may be of any desired cross-section, e.g., circular, polygonal, or other shape. The **monolithic** matrix may be polymerized in situ within the tube, or it may be formed outside of the tube and then inserted by any suitable means.

DEPR:
In alternative embodiments, the **monolithic** matrix is (i) held in a channel or groove on a plate, or (ii) applied as a thin film across a plate. While the **monoliths** of the invention may be provided on a substrate or supporting plate of virtually any size and composed of any suitable material, one embodiment of the present invention contemplates using a supporting plate of a standard size, which can be constructed using conventional materials and means. Thus, in this embodiment, an injection molded rectangular plastic plate, the length and width of which conform to the commonly used standard of 5.030".times.3.365" (127.76 mm and 85.47 mm), is preferred. Similarly, while a single plate may support any reasonable number of sites, constructions corresponding to the commonly used "96-well" microtiter plate format are preferred. Thus, one preferred embodiment includes an 8.times.12 array of sites situated on a standard-sized support plate. Each site may support one or more separate **monoliths**. The position and spacing of the sites on the support plate may be standard, as well (e.g., spaced about 9 mm center-to-center). Utilization of standard outside dimensions for the plate frame, as well as standard spacing for

the sites on
the plate, facilitates use of the plates with existing equipment, such as
automated dispensers or optical readers, if desired. It should be appreciated
that such an apparatus is capable of handling multiple simultaneous and
parallel separations.

DEPR:
In a further embodiment, the **monolithic** matrix fills one or more channels
formed on a microchip. For example, a plurality of channels can be formed on a
glass microchip substrate using standard microfabrication techniques, e.g.,
photolithographic procedures and chemical wet etching. Optionally, a cover
plate can be direct bonded to the substrate over the channels. A plurality of
separations can be conducted on multiple samples in a substantially parallel
fashion upon a single substrate chip.

DEPR:
Advantageously, the columns of this invention are not limited to the fixed
surface areas, pressure drops and interstitial diffusional distances of a
packed bed. Because the void fraction can be much higher in the **monolithic**
columns taught herein, all of these factors can be manipulated for improved
performance for a given application. For example, columns can be produced with
the same void fraction and widely different pressure drops, or columns could be
produced with the same pressure drop but different interstitial distances. In
a particular example, some columns produced in this invention [illegible]
for DNA separations have void fractions of about 0.7. A void [illegible]
cannot be produced for nonporous packings by packing [illegible]
particles.

DEPR:
FIG. 1 is a chromatogram showing the separation of single-stranded
oligothymidylic acids on a C6 **monolithic** column constructed in

the manner just
described. Specifically, the sample was 10 microliters of oligothymidylic adds
between 12 and 18 units in length. At a flow rate of 2 ml/minute, a gradient
of 0-12% acetonitrile in 100 mM aqueous triethylammonium acetate, 10 mM
disodium EDTA, pH 7.0 was used to elute the oligonucleotides. Detection was
performed using UV absorbance at 254 nm. An extremely low HPLC system pressure
of only 150 psi was observed during the separation.

DEPR:
FIG. 2A is a chromatogram showing the separation of double-stranded DNA
fragments on a **monolithic** C12 column constructed in the manner just described.
Specifically, the sample was 3 microliters of pUC18 DNA digested by the MSP I
restriction enzyme. At a flow rate of 1.0 ml/minute, a 10 minute gradient of
35-60% acetonitrile containing 2 mM tetrapropylammonium bromide in 20 mM
aqueous tetrapropylammonium bromide, 2 mM disodium EDTA, pH 7.0 was used to
elute the DNA fragments. Detection was performed using UV absorbance at 254
nm. At 0.5 ml/min an HPLC system pressure of 50 psi was observed. Resolution
was calculated for the peaks indicated in FIG. 2A by arrows.

DEPR:
The chromatographic data from the above experiments are summarized in Table 1.
The resolution of the marked peaks was found to be 4.04 for the **monolithic**
column of FIG. 2A and 5.83 for the column packed with nonporous spheres in FIG.
2B. Using standard permeability calculations (Introduction to Modern Liquid
Chromatography, 2.sup.nd edition, L. R. Snyder and J. J. Kirkland, John Wiley
and Sons, New York, 1979, p. 77), it can be calculated that the column in FIG.
2A has an operating pressure equivalent to that of a column packed with 6.2
micron diameter spheres whereas that in FIG. 2B would be estimated as 1.70
micron. Based on those different effective partide sizes, the

column of FIG.
2A has a resolution 58% greater than expected. A critical figure of merit for
chromatographic separations is the efficiency or resolution per unit pressure.
It can be seen from the data of Table 1 that the **monolithic** column offers an
order of magnitude increase in resolution per unit pressure compared to the
column packed with nonporous spheres.

DEPR:
The present invention also makes possible the separation of partially denatured
double-stranded polynucleotides. As shown in FIGS. 3A and 3B, a porous
monolithic column was used to separate DNA fragments 304 base pairs in length.
In FIG. 3A, the sample contained a single type of DNA, whereas in FIG. 3B the
sample contained a mixture of the type seen in FIG. 3A and a variant sequence
containing a single base pair substitution. At the selected temperature, the
sample of FIG. 3B shows a second peak that represents partially denatured DNA
containing the single base pair substitution.

DEPR:
Specifically, with regard to FIG. 3A, homoduplex DNA with sequence 304 base
pairs in length was separated on a **monolithic** C12 column, prepared as described
above. In carrying out the separation, the conditions as set out in Table 2,
below, were observed:

DEPR:
For comparative purposes, columns were constructed in accordance with Examples
III and VI of the '310 patent. Briefly, these examples describe **monolithic**
[illegible] polymerizations of these
compositions, end fittings were attached and the columns flushed with methanol,
as described in the '310 patent. The composition produced by Example III of
the '310 patent was translucent in appearance and had a very high operating

pressure (greater than 2,000 psi at 0.25 ml/min with methanol), precluding its
use in practical HPLC of DNA. The composition produced by Example VI of the
'310 patent was white and had a relatively low operating pressure. However,
this latter column did not provide useful separations of DNA restriction
fragments under reversed-phase ion-pairing conditions. No typical pattern of
peaks was observed when a separation was attempted under conditions that would
provide a useful separation using the matrices of the present invention. These
findings are in accord with previous suggestions in the literature that columns
with unmodified polystyrene/divinylbenzene structures are not desirable for DNA
separations.

DETL:
TABLE 1 Column with Nonporous Column with C12 Spherical Backing **Monolithic**
Polymer Retention Time Peak 1 (min.) 6.80 9.36 Area Peak 1 (.mu.V*sec)
80678.00 84592.00 Height Peak 1 (.mu.V) 11370.00 7436.00 Retention Time Peak
2 (min.) 7.52 10.10 Area Peak 2 (.mu.V*sec) 103115.00 103644.00 Height Peak 2
(.mu.V) 13348.00 9779.00 Resolution 5.83 4.04 Column Pressure (psi) 1340.00
100.00 Apparent Particle Size (.mu.m) 1.20 6.20 Expected Resolution 5.83 3.57
Excess Resolution 0.00 58.00 Resolution/1,000 psi 4.35 40.42

CLPR:
2. The method of claim 1, wherein said separating comprises passing an eluant
containing an ion-pairing agent through said **monolithic** matrix, said
ion-pairing agent being an alkylammonium salt.

CLPR:
4. The method of claim 1, further comprising the step of passing an eluant
containing an ion-pairing agent through said **monolithic** matrix, said
ion-pairing agent being capable of interacting with negatively charged
phosphate groups of said at least one polynucleotide and also

with said
hydrophobic surface of said **monolithic** polymer matrix.

CLPP:
9. The method of claim 8, wherein said **monolithic** polymer matrix is held in a
channel formed in said plate.

CLPR:
10. The method of claim 8, wherein said **monolithic** polymer matrix takes the
form of a thin film on said plate.

CLPV:
applying said mixture to a porous **monolithic** polymer matrix held in a
stationary fashion by a support,

CLPV:
wherein said **monolithic** polymer matrix is formed from polymerization of a
monomer, or combination of monomers, selected from C.sub.3 to C.sub.30 alkyl
methacrylates, in the presence of a crosslinking agent and a pirogenic solvent,
and

CLPV:
separating said mixture by ion-pair reverse-phase chromatography, by passing an
eluant containing an ion-pairing agent through the **monolithic** matrix.

CCXR:
210/198.2

OREL:
Article by Viklund et al, entitled "`Molded` Macroporous Poly (glycidlyimethacrylate-co-trimethylolpropane trimethacrylate), Materials with
Fine Controlled Porous Properties: Preparation of **Monoliths** Using Photoinitiated Polymerization" published in Chem Mater. In 1997, [illegible]
[illegible].

OREL:
Article by Viklund et al., entitled "**Monolithic,** Molded , Porous Materials
with High Flow Characteristics for Separations, Catalysis, or Solid-Phase

Chemistry: Control of Porous Properties during Polymerization," published in
Chem. Mater in 1996, in vol. 8, pp. 744-750.

DOCUMENT-IDENTIFIER: US 6218153 B1
TITLE: Target DNA amplification by MIPC and PCR

DEPR:
MIPC uses unique non-polar separation media which comprises organic polymers, silica media having a non-polar surface comprising coated or covalently bound organic polymers or covalently bound alkyl and/or aryl groups, and continuous non-polar separation media, i.e., **monolith** or rod columns such as non-polar silica gel or organic polymer. The separation media used in MIPC can be porous or non-porous. A detailed description of the MIPC separation process, MIPC separation media, and MIPC systems is found in U.S. Pat. No. 5,772,889 (1998) to Gjerde and in co-pending U.S. patent applications Ser. Nos. 09/066,580 filed Mar. 10, 1998; (abandoned); 09/058,337 filed Mar. 10, 1998; (abandoned); 09/061,040 filed May 13, 1998 (now U.S. Pat. No. 5,947,742); 09/080,547 filed May 18,1998 (now U.S. Pat. No. 6,017,457); and in the U.S. patent application Ser. No. 09/169,440 filed Oct. 9, 1998. MIPC systems and separation media are commercially available (Transgenomic, Inc. San Jose, Calif.). The entire MIPC analysis can be automated by means of a desk top computer and a sample auto-injector. Analytical data for each sample can be analyzed in real time, or collected and stored in a computer memory device for analysis at a later time.

CCLR:
210/198.2

DOCUMENT-IDENTIFIER: US 6210885 B1
TITLE: Modifying double stranded DNA to enhance separations by matched ion
polynucleotide chromatography

BSPR:
In one aspect, the invention provides a method for enhancing the detection of a
polynucleotide separated by Matched Ion Polynucleotide Chromatography which
includes (a) covalently attaching a chemical tag to the polynucleotide to form
a tagged polynucleotide, (b) applying the tagged polynucleotide to a separation
medium having a non-polar separation surface, the medium characterized by
having a DNA Separation Factor of at least 0.05, (c) eluting the tagged
polynucleotide from the surface with a mobile phase containing a counterion
agent and an organic solvent, and (d) detecting the tagged polynucleotide. The
chemical tag is preferably a fluorescent group, a chemical which absorbs at a
wavelength different from the polynucleotide itself, or, less preferably, a
group containing a radioactive atom (e.g., P-32, tritium, or S-35).
Non-limiting examples of fluorescent groups which absorb at a wavelength
different from the polynucleotide itself include
6-carboxyfluorescein,
3',7'-dimethoxy-4',5'-dichloro-6-carboxyfluorescein,
N,N,N',N'-tetramethyl-6-carboxyrhodamine, 6-carboxy-X-rhodamine,
Fluorescein,
Rhodamine, BODIPY-TR-X, Cascade Blue, Alexa 350, and porphyrin derivatives
(e.g., TPP4py+). Non-limiting examples of fluorescent groups which absorb
[illegible]
',7'-dimethoxy-4',5'-dichloro-6-carboxyfluorescein,
N,N,N', N'-tetramethyl-6-carboxyrhodamine, 6-carboxy-X-rhodamine,
Fluorescein,
Rhodamine, BODIPY-TR-X, Cascade Blue, and Alexa 350. The preferred separation
medium is characterized by having a Mutation Separation Factor of at least 0.1.
The preferred medium is substantially free from contamination

with multivalent
cations. In one embodiment, the separation medium consists of polymer beads
having an average diameter of 0.5 to 100 microns and having a surface
composition essentially completely substituted with a moiety selected from the
group consisting of unsubstituted, methyl, ethyl, hydrocarbon, and hydrocarbon
polymer, wherein the hydrocarbon polymer optionally has from 23 to 1,000,000
carbons, wherein the hydrocarbon includes alkyl and alkyl substituted aryl
groups having from 23 to 1,000,000 carbons, the alkyl groups including straight
chained, branch chained, cyclic, saturated, unsaturated nonionic functional
groups of various types including aldehyde, ketone, ester, ether, alkyl groups,
and the like, and the aryl groups including as monocyclic, bicyclic, and
tricyclic aromatic hydrocarbon groups including phenyl, naphthyl, and the like.
In another embodiment, the separation medium consists of beads having an
average diameter of 0.5 to 100 microns, the beads comprising nonporous
particles coated with a hydrocarbon or non-polar hydrocarbon substituted
polymer, wherein the hydrocarbon has optionally from 1 to 1,000,000 carbons,
wherein the hydrocarbon polymer has optionally from 1 to 1,000,000 carbons, or
particles having substantially all polar groups reacted with a non-polar
hydrocarbon or substituted hydrocarbon group, wherein the particles are a
member selected from the group consisting of silica, silica carbide, silica
nitrite, titanium oxide, aluminum oxide, zirconium oxide, carbon, [illegible]
polystyrene, and diatomaceous earth. In other embodiments, [illegible]
medium is a polymeric **monolith** or a derivatized silica gel **monolith**. The
tagged polynucleotide can be a PCR amplification product obtained by providing
a PCR primer having a covalently bound tag during a PCR amplification wherein
the tag is incorporated into the PCR amplification product.

DEPR:
The medium can be enclosed in a column. In one embodiment, the non-polar surfaces comprise the surfaces of separation beads, such as polymeric beads or derivatized particles (e.g., silica particles). In an alternative embodiment, the surfaces comprise the surfaces of interstitial spaces in a molded **monolith** such as a polymeric **monolith** or a silica gel **monolith**. For purposes of simplifying the description of the invention and not by way of limitation, the separation of polynucleotides using nonporous beads, and the preparation of such beads, will be primarily described herein, it being understood that other separation surfaces, such as the surfaces within interstitial spaces of **monoliths,** are intended to be included within the scope of this invention. Examples of suitable **monoliths** include polymeric rods and derivatized silica gel rods which have been formed inside a column as a unitary structure having through pores or interstitial spaces which allow eluting solvent and analyte to pass through and which provide the non-polar separation surface.

DEPR:
In another embodiment of the present invention, the separation medium can be in the form of a polymeric **monolith** such as a rod-like **monolithic** column. The **monolithic** column is polymerized or formed as a single unit inside of a tube. The through pore or interstitial spaces provide for the passage of eluting solvent and analyte materials. The separation is performed on the stationary surface. The surface can be porous, but is preferably nonporous. Elution of the separations are identical to columns packed with beads. As with beads, the pores contained in the rod must be compatible with DNA and not trap the material. Also, the rod must not contain contamination that will trap DNA.

DEPR:
The molded polymeric rod of the present invention is prepared by bulk free radical polymerization within the confines of a chromatographic column. The base polymer of the rod can be produced from a variety of polymerizable monomers. For example, the **monolithic** rod can be made from polymers, including mono- and di-vinyl substituted aromatic compounds such as styrene, substituted styrenes, alpha-substituted styrenes and divinylbenzene; acrylates and methacrylates; polyolefins such as polypropylene and polyethylene; polyesters; polyurethanes; polyamides; polycarbonates; and substituted polymers including fluorosubstituted ethylenes commonly known under the trademark TEFLON. The base polymer can also be mixtures of polymers, non-limiting examples of which include poly(glycidyl methacrylate-co-ethylene dimethacrylate), poly(styrene-divinylbenzene) and poly(ethylvinylbenzene-divinylbenzene. The rod can be unsubstituted or substituted with a substituent such as a hydrocarbon alkyl or an aryl group. The alkyl group optionally has 1 to 1,000,000 carbons inclusive in a straight or branched chain, and includes straight chained, branch chained, cyclic, saturated, unsaturated nonionic functional groups of various types including aldehyde, ketone, ester, ether, alkyl groups, and the like, and the aryl groups includes as mononcyclic, bicyclic, and tricyclic aromatic hydrocarbon groups including phenyl, naphthyl, and the like. In a preferred embodiment, the alkyl group has [illegible]. In a more preferred embodiment, the alkyl group has 1-8 carbons. [illegible] can also contain hydroxy, cyano, nitro groups, or the like which are considered to be non-polar, reverse phase functional groups. Methods for hydrocarbon substitution are conventional and well-known in the art and are not an aspect of this invention. The preparation of polymeric **monoliths** is by

conventional
methods well known in the art as described in the following
references: Wang et
al. (J. Chromatog. A 699:230 (1994)), Petro et al. (Ana. Chem.
68:315
(1996)), and the following U.S. Pat. Nos. 5,334,310;
5,453,185; 5,522,994
(to Frechet). **Monolith** or rod columns are commercially available
form Merck &
Co (Darmstadt, Germany).

DEPR:
In another embodiment of the present invention, the separation
medium is
continuous **monolithic** silica gel. A molded **monolith** can be
prepared by
polymerization within the confines of a chromatographic column
(e.g., to form a
rod) or other containment system. A **monolith** is preferably
obtained by the
hydrolysis and polycondensation of alkoxysilanes. A preferred
monolith is
derivatized in order to produce non-polar interstitial surfaces.
Chemical
modification of silica **monoliths** with octadecyl, methyl or other
ligands can be
carried out. An example of a preferred derivatized **monolith** is
one which is
polyfunctionally derivatized with octadecylsilyl groups. The
preparation of
derivatized silica **monoliths** is by conventional methods well
known in the art
as described in the following references which are hereby
incorporated in their
entirety herein: Nakanishi, et al., J. Sol-Gel Sci. Technol.
8:743 (1997);
Nakanishi, et al., Bull, Chem. Soc. Jpn. 67:1327 (1994);
Cabrera, et al.,
Trends Analytical Chem. 17:50 (1998); Jinno, et al.,
Chromatographia 27:288
(1989).

CLPV:
(c) detecting said tagged polynucleotide, wherein said medium is
characterized
by having a DNA Separation Factor of at least 0.5, wherein said
medium
comprises a polymeric **monolith**.

CLPV:

d) detecting said tagged polynucleotide, wherein said medium is characterized
by having a DNA Separation Factor of at least 0.5, wherein said medium
comprises a derivatized silica gel **monolith**.

CCXR:
210/198.2

DOCUMENT-IDENTIFIER: US 6210570 B1
TITLE: **Monolithic** silica column

TTL:
Monolithic silica column

ABPL:
The present invention relates to capillary columns including a **monolith** and a
method for preparing a capillary column including a **monolith**. The **monolith** can
be prepared by a sol gel method, and in the transformation from hydrosol to
hydrogel, the **monolith** undergoes essentially no syneresis or volume shrinkage.
Thus, deleterious effects of syneresis are avoided, such as the formation of
channels having large dimensions that provide a pathway of least resistance for
a mobile phase to effectively bypass portions of a stationary phase. The
method for preparing a column having a **monolith** that undergoes essentially no
syneresis involves a hydrogel solution that has a relatively low concentration
of SiO.sub.2, i.e. less than about 5 g/100 mL.

BSPR:
The present invention relates to a method for producing chromatography columns,
in particular a silica column, via a sol-gel method. The initial hydrosol has
a composition featuring less than 5% SiO.sub.2 to produce a hydrogel that
undergoes essentially no syneresis. The present invention also encompasses a
capillary chromatography column including a novel **monolith**.

BSPR:
In recent [illegible] phases
comprising a continuous network have been developed. This continuous network
phase or **monolith** can comprise pores of an appreciable dimension and at the
same time, eliminate gaps or channels that can arise from poorly packed

columns. For example, U.S. Pat. No. 5,624,875 relates to methods for preparing inorganic porous materials having pores of various desired dimensions.

BSPF:
The invention provides capillary column incorporating a **monolith,** and also a method for making the chromatography column.

BSPF:
One aspect of the invention provides a method for preparing a chromatography column. The method includes the step of providing an aqueous mixture including a compound having at least one hydrolyzable oxygen-containing group. The method also involves causing the mixture to form a hydrosol via a reaction involving the at least one hydrolyzable oxygen-containing group. The hydrosol is introduced into a capillary, the hydrosol having a first volume. Gellation of the hydrosol is induced to produce a **monolith**. The **monolith** has a second volume, wherein the second volume is at least about 95% of the first volume.

BSPF:
One embodiment of the invention provides a method for preparing a chromatography column. The method involves an aqueous mixture of a compound having at least one hydrolyzable oxygen-containing group formed into a hydrosol via a reaction involving the at least one hydrolyzable oxygen-containing group, the hydrosol being positioned in a capillary. The improvement comprises the hydrosol being selected to have a first volume, the hydrosol being induced to gel to produce a **monolith**. The **monolith** has a second volume, wherein the second volume is at least about 95% of the first volume.

BSPF:
Another aspect of the invention provides a capillary column. The column has a porous **monolith, the monolith** having pores of a first mean diameter and

channels of a second mean diameter. The second mean diameter is greater than
the first mean diameter by less than about 150% of the first mean diameter.

PSPP:
One embodiment provides a capillary column including a **monolith** wherein the
column is free of deleterious effects due to syneresis.

DRPP:
FIG. 3A is a schematic illustration of a cross-sectional slice of a capillary
column containing a **monolith** support in which the **monolith** has undergone
essentially no syneresis;

DRPP:
FIG. 3B is a schematic illustration of a cross-sectional slice of a capillary
column containing a **monolith** support in which the **monolith** has undergone
syneresis;

DRPP:
FIG. 4A is a schematic cross-sectional area of a capillary column containing a
monolith support in which the **monolith** has undergone essentially
no syneresis;

DRPP:
FIG. 4B is a schematic cross-sectional area of a capillary column containing a
monolith support in which the **monolith** has undergone syneresis
and featuring
the different types of channels in the column;

DRPP:
FIG. 5A is a photocopy of an electron micrograph of a silica
monolith prepared
[illegible]
distance of 3[illegible]
[illegible]

DETD:
The present invention relates to chromatography columns and reactants and
reactant concentrations for preparing stationary phase materials that undergo
essentially no syneresis. In particular, the stationary phase of

the column
comprises a continuous network, such as a **monolith**.

DEPF:
Chromatography columns preferably comprise a high surface area, stationary
phase material. Because separation of components is achieved by differentiating adsorption/desorption rates of the components as they traverse
along the stationary phase, a high surface area material can minimize a total
volume of the stationary phase. High surface areas can be achieved with porous
materials. In the previously described hydrosol formation process, porous
silica can be formed by the addition of a polymer to the initial pre-hydrosol
mixture. The polymer also forms a continuous network and the polymer network
is interconnected or interspersed with the network of the hydrosol and
eventually the hydrogel. The polymer can either be added as a separate entity
or can be generated during the hydrolysis reaction. Preferably, the polymer is
a low molecular weight polymer having an average molecule weight of between
about 1,000 g/mol and about 50,000 g/mol, more preferably between about 1,000
g/mol and about 30,000 g/mol, more preferably between about 5,000 g/mol and
about 20,000 g/mol. The polymer can be present preferably in an amount of
between about 0.05 g/mL and about 0.5 g/mL, more preferably between about 0.075
g/mL and about 0.3 g/mL. The polymer preferably has desirable characteristics
of non-toxicity, hydrophilicity and solubility in the solution and can be
either ionic or nonionic. In one embodiment, the polymer is an [illegible]
such as poly(sodium styrenesulfonate) or poly(potassium [illegible]. In
another embodiment, the polymer can be a nonionic polymer such as polyethylene
glycol. The polymer can be removed or eluted prior to chromatography by
rinsing with an appropriate solvent, such as water and/or alcohol. The column
may be further prepared by methods such as supercritical drying

or by the use
of a reagent to coat the gel with hydrophobic groups (e.g. methyl groups) to
maintain hydrolytic stability. The **monolith** can also be stored with the
polymer network interspersed within.

DEPR:
The method also involves inducing gellation of the hydrosol to form the
hydrogel. In one embodiment, the hydrogel is a **monolith** i.e. a solid
comprising a continuous network of chemical bonds. Gellation can be induced in
a number of ways known in the art. In one embodiment, gellation is induced by
warming an aqueous mixture comprising alkoxysilanes and a catalyst. In another
embodiment, gellation can be induced by warning the hydrosol. Gellation can be
induced at temperatures between about 0.degree. C. and 70.degree. C. In
another embodiment, the solution can be warmed to temperatures of between about
[illegible] and about 70.degree. C., preferably between about 30.degree.
C. and about 60.degree. C., and more preferably between about 30.degree. C.
and about 50.degree. C. In another embodiment, gellation can be induced by
allowing the mixture to stand at a temperature of between about 20.degree. C.
and about 30.degree. C. It is understood that the optimal temperature is
dependent on reactant concentration, pH etc.

DEPR:
A particularly advantageous feature of the invention involves the formation of
a **monolith** product where the hydrosol to gel transformation
[illegible]
essentially no syneresis. "Syneresis" is the shrinkage in volume
[illegible]
progresses to a hydrogel. During both hydrosol and hydrogel
[illegible],
are formed to generate a larger network without any decrease in volume. Bond
formation can include hydrogen bonding or condensation reactions as discussed
previously. As the gellation process continues and the network

increases in volume, there reaches a point when bond formation results in a shrinkage of the network i.e. bond formation between two atoms causes several atoms to shift positions spatially such that the shifted atoms encompass a smaller local area or local volume. For example, FIG. 1A is a schematic illustration of two surfaces 10 each having a hydrolyzable hydroxide group 12. FIG. 1B schematically depicts the product of hydrolysis reaction between the two hydroxide groups to forms linkage 14, resulting in the two surfaces 10 being forced into closer proximity to each other. Thus, atoms at or near surface 10 shift in response to formation of linkage 14. The result may be a smaller local volume, as depicted schematically in FIG. 1B. In another example, a molecular representation of a surface of silica is shown in FIG. 2, the surface having hydrolyzable hydroxide groups 16. A hydrolysis reaction between any two groups 16 can cause at least a decrease in local volume and a resulting decrease in a total volume of the silica. Syneresis can be irreversible, the reversibility dependent on the ease of bond breaking.

DEPR:
Another embodiment of the present invention involves the formation of a capillary column having a **monolith** stationary phase. Conventional capillary columns comprise a cylindrical article having an inner wall and an outer wall and involve a stationary phase permanently positioned within a circular [illegible] having inner diameters ranging from 5 .mu.m to 0.5 mm. The [illegible] of the tube can have various closed shapes corresponding to the [illegible] of the cylinder tube. The tube wall is preferably glass but can be made of metal, plastic and other materials. Typically, the stationary phase comprises particles that are permanently packed adjacent the

inner wall by various high pressure processes well known to those of ordinary skill in the art. The present invention of a **monolith** capillary column features an advantage over conventional capillary column due to facile preparation precluding the high pressure conditions. In particular, facile conditions are desired for smaller diameter capillaries (e.g. less than 100 .mu.m diameter) where particle packing presents added difficulties due to the small dimensions.

DEFR:
The ability to prepare a **monolith** without syneresis is an important feature in preparing **monolith** capillary columns. FIG. 3A shows a schematic example of a cross-sectional slice of an ideal capillary column 20 including capillary walls 22 and a **monolith** 24. During chromatography, arrows 26 show a pathway traversed by the mobile phase, the pathway maximizing contact between the mobile and stationary phases. FIG. 3B shows a schematic example of a cross-sectional slice of a capillary column 30 after syneresis, the column having a capillary wall 32 and a shrunken **monolith** 34. Due to a shrinkage in volume, channels can be formed between the **monolith** 34 and wall 32 or within the **monolith**. During chromatography, a flow path of least resistance exists within these gaps. A mobile phase traversing along this column may tend to follow the pathway indicated by arrows 36 instead of desired pathway 38. When the mobile phase follows pathways 36, portions of the stationary phase can be bypassed. Streaking of components to be separated can occur through the **monolith** and optimal separation of components may not be achieved. Due to factors such as temperature, hydrosol composition or even the application of an electric field, syneresis can occur to the extent that a volume of a material can decrease by a factor of 100.

DEPR:
Thus, another embodiment of the invention provides a capillary column having a **monolith** that is essentially free of syneresis. In one embodiment, the hydrosol is introduced into the capillary column, the hydrosol having a first volume. The first volume can be the volume of the hydrosol as defined by boundaries of the capillary. Gellation of the hydrosol can then be induced to form the **monolith, the monolith** having a second volume defined as the entire volume encompassed by the outer boundaries of the **monolith**. The second volume is at least about 95% of the first volume. Preferably, the second volume is at least about 99% of the first volume.

DEPR:
For silica **monoliths,** it has been discovered that producing a hydrosol having a relatively low ratio of SiO.sub.2 units to solution volume results in a solid silica material that has undergone essentially no syneresis, as discussed in Jones et al. J. Non-Crystalline Solids, Vol. 101, pp. 123-126 (1988). In one embodiment, the hydrosol has an SiO.sub.2 concentration of less than about 5 g/mL, preferably between about 3 g/mL and about 5 g/mL and more preferably between about 4 g/mL and about 5 g/mL. A balance should be achieved between preventing syneresis and reducing the structural integrity of the silica material which can be caused by an extremely low SiO.sub.2 concentration. A further decrease in SiO.sub.2 concentration may reduce a number of silica [illegible] units and a brittle silica product may result.

DEPR:
In another embodiment, gellation is induced inside a capillary to provide a capillary column comprising a **monolith**. An advantageous feature of inducing gellation inside a capillary is the possibility of a covalent attachment

between a capillary inner wall and the monolith, providing the column with a structural integrity that maintains the monolith within the column. A covalent attachment refers to the formation of a covalent chemical bond between the monolith and the capillary. For example, the capillary can be made of glass. A surface of the glass, preferably the inner wall of the glass capillary, can have condensible chemical groups. In one embodiment, the groups can be terminal Si--OH groups which can undergo a condensation reaction with the monolith which also has condensible chemical groups. For example, the monolith can have terminal M--OH groups which can react with Si--OH groups of the inner capillary wall to produce a covalent M--O--Si linkage between the monolith and the capillary. In one embodiment, M of the monolith can be any metal or main group element, such as silicon to provide an Si--O--Si linkage.

DETA:
To allow the mobile phase to pass through the monolith, preferably the monolith is a porous monolith having pores of an average pore dimension or diameter. Preferably the average pore dimension is between about 0.1 .mu.m and about 10 .mu.m, and more preferably between about 0.25 .mu.m and about 5 .mu.m.

DETA:
When there is a covalent attachment between the inner capillary wall and the monolith, the capillary column can have also have pores defined by a portion of the monolith, a portion of the wall and the covalent bonds. Such pores in a capillary column that undergoes essentially no syneresis have [illegible] comparable to the pores of the monolith. FIG. 4A shows a schematic cross-sectional area of a capillary column having a monolith that is formed with essentially no syneresis. Capillary column 40 has a capillary 41 comprising an inner wall 42. Monolith 44 is attached to inner

wall 42.
Monolith 44 also has pores, for example the cross-section of pore 46, which
have a first mean diameter.

DEPR:
FIG. 4B schematically illustrates a cross-sectional area of a capillary column
50 having a **monolith** that is formed with syneresis. Syneresis causes a
shrinkage in volume of the **monolith** resulting in deleterious effects such as
the formation of channels. These channels can have excessively large
dimensions that can provide, at least in part, a flow path of least resistance
for portions of the mobile phase to bypass portions of the stationary phase.
The extent of absorption/desorption of the mobile phase is minimized and
streaking of the component bands can occur, resulting in poor resolution. In
FIG. 4B, channels such as channel 48a can be formed when syneresis causes bonds
between the **monolith** and the wall to break. In addition, channels may be
formed within the **monolith**, such as channel 48b.

DEPR:
Another embodiment provides a capillary column having a silica **monolith** where
channels have a second mean diameter, such that the second mean diameter is
greater than the first mean diameter (of the pores) by less than about 150% of
the first mean diameter, preferably about 125% of the first mean diameter, more
preferably less than about 110% of the first mean diameter, more preferably
less than about 105% of the first mean diameter, and even more preferably less
than about 101% of the first mean diameter.

DEPR:
In another embodiment, the method allows further derivatization of the
monolith. This derivatization allows tailoring of the **monolith** for a variety
of chromatographic separations. For example, a surface can be incorporated

into the monolith that is useful for reverse phase chromatography. Such surfaces can comprise long chain alkyls or other nonpolar groups. If, for example, the monolith is silica, the surface may include Si--OH or Si--OR groups that can be derivatized to form other Si--O-linkages to other organic groups, such as alkyls. Other derivatizations are known in the art and these are in accordance with the method of the invention.

DEPR:
Another aspect of the invention provides a capillary column having a monolith. The monolith can have features described above of a monolith prepared in accordance with the method of the present invention, including reaction conditions such as reactants and reactant concentration. In particular, the monolith of the capillary column is prepared with essentially no syneresis to avoid the formation of undesired channels that may result in poor chromatographic performance.

DEPR:
A silica monolith column can also be prepared by following the above procedure but substituting 887 .mu.L of tetraethylorthosilicate for the tetramethylorthosilicate.

DEPC:
Example: Preparation of a Silica Monolith Column

CLPV:
a porous silica monolith having pores of a first mean diameter and channels of a second mean diameter, wherein the second mean diameter is greater than the first mean diameter by less than about 10% of the first mean diameter, wherein

CCOR:
210/198.2

DOCUMENT-IDENTIFIER: US 6136187 A
TITLE: Separation column containing porous matrix and method of packing column

DEPR:
With this **monolithic** packing method, chromatographic materials that are charged and uncharged in nature can be embedded into the sol-gel matrix. Different functionalized/derivatized sol-gel precursors can be used to prepare sol-gel glasses with different physical properties, such as pore size and surface charge. The pore size may be selected by choosing an appropriate sol-gel precursor. For example, to obtain larger pores, tetramethylorthosilicate may be used as the precursor instead of tetraethylorthosilicate indicated above.

CCOR:
210/198.2

ORPL:
"Preparation and Characterization of **Monolithic** Porous Capillary Columns Loaded with Chromatographic Particles," M. Dulay et al., Anal. Chem. vol. 70, No. 23, Dec. 1, 1998, pp. 5103-5107.

DOCUMENT-IDENTIFIER: US 6077434 A
TITLE: Current-efficient suppressors and method of use

DEPR:
The term "packing" refers to stationary flow-through solid material disposed in a flow channel of the suppressor. It can be a screen or a porous **monolithic** matrix, a resin particle bed or other form. It can be strongly charged, weakly charged or of neutral charge, as will be explained. The term packing is alternatively called "bridging means."

DEPR:
In the above system, one way to increase current efficiency is leave the sample stream flow channel open without packing or to use packing which is of neutral charge or of low capacity relative to the packing of high capacity ion exchange material in the ion receiving flow channel and, for a two membrane suppressor, in the ion source channel. While the above description refers to the stationary flow-through packing of ion exchange material in the form of a high capacity charged screen, other forms of packing may also be employed as described above. Such other packing forms of ion exchange material include packed beds of ion exchange resin or **monolithic** materials of charged material with sufficient porosity for the flow of an aqueous liquid stream through them. The packing in the ion receiving channel has a substantially higher capacity than ion exchange packing in the sample flow channel, if present. Thus, if a charged screen is used in the sample stream flow channel, it preferably is of low capacity, with a capacity of substantially less than that of the packing in the ion receiving flow channel. Suitably, the ratio of total capacities of the packing in the sample stream flow channel to that in the ion receiving stream

flow channel is no greater than about 0.9, and preferably no greater than about
0.7 to 0.5, and more preferably no greater than about 0.1.

CCXR:
210/198.2

DOCUMENT-IDENTIFIER: US 6071410 A
TITLE: Recovery of organic solutes from aqueous solutions

BSPR:
In an alternative embodiment, the sorbent bed is a **monolithic** matrix formed in,
or inserted into, an appropriate supporting structure, such as a column tube,
cartridge, microplate well, or channel of a microdevice.

CCXR:
210/198.2

DOCUMENT-IDENTIFIER: US 6066258 A
TITLE: Polynucleotide separations on polymeric separation media

ABPL:
Non-polar polymeric separation media, such as beads or **monoliths,** are suitable
for chromatographic separation of mixtures of polynucleotides when the surfaces
of the media are unsubstituted or substituted with a hydrocarbon group having
from one to 1,000,000 carbons and when the surfaces are substantially free from
multivalent cation contamination. The polymeric media provide efficient
separation of polynucleotides using Matched Ion Polynucleotide Chromatography.
Methods for maintaining and storing the polymeric media include treatment with
multivalent cation binding agents.

BSPR:
The present invention is directed to the separation of polynucleotides using
non-polar separation surfaces, such as the surfaces of polymeric beads and
surfaces within molded **monoliths,** which are substantially free from
contamination with multivalent cations.

BSPR:
Another object of the present invention is to provide a method for separating
polynucleotides using nonporous polymer separation media, such as beads or
monoliths (e.g., rods), having non-reactive, non-polar surfaces.

BSPR:
In one aspect, the invention is a method for separating a mixture of
polynucleotides by applying a mixture of [illegible] pairs to a polymeric
separation medium having non-polar surfaces which are
substantially free from contamination with multivalent cations, and eluting the
mixture of polynucleotides. The preferred surfaces are nonporous. The

non-polar surfaces can be enclosed in a column. In the preferred embodiment, precautions are taken during the production of the medium so that it is substantially free of multivalent cation contaminants and the medium is treated, for example by an acid wash treatment and/or treatment with multivalent cation binding agent, to remove any residual surface metal contaminants. The preferred separation medium is characterized by having a DNA Separation Factor (defined hereinbelow) of at least 0.05. The preferred separation medium is also characterized by having a Mutation Separation Factor (as defined hereinbelow) of at least 0.1. In the preferred embodiment, the separation is made by Matched Ion Polynucleotide Chromatography (MIPC, as defined hereinbelow). Examples of non-polar surfaces include the surfaces of polymer beads and the surfaces of interstitial spaces within a polymeric **monolith**. The elution step

BSPR:
In yet another embodiment, the invention is a method for separating a mixture of polynucleotides comprising flowing a mixture of polynucleotides having up to 1500 base pairs through a polymeric **monolith,** and separating the mixture of polynucleotides using MIPC. In this embodiment, the non-polar separation surfaces are the surfaces of interstitial spaces of a polymeric **monolith**. An example of such a **monolith** is a polymeric rod prepared within the confines of a chromatographic column. The **monolith** of the invention is characterized by having a DNA Separation Factor of at least 0.05. In a preferred embodiment, the **monolith** is characterized by having a DNA Separation Factor of at least 0.5. The **monolith** is preferably characterized by having a Mutation Separation Factor of at least 0.1. The mobile phase used in the separation preferably includes an organic solvent as exemplified by alcohol, nitrile,

dimethylformamide, tetrahydrofuran, ester, ether, and mixtures thereof.
Examples of suitable solvents include methanol, ethanol, 2-propanol,
1-propanol, tetrahydrofuran, ethyl acetate, acetonitrile, and mixtures thereof.
The most preferred organic solvent is acetonitrile. The mobile phase
preferably includes a counterion agent such as lower primary, secondary and
tertiary amines, and lower trialkyammonium salts, or quaternary ammonium salts.
More specifically, the counterion agent can be octylammonium acetate,
octadimethylammonium acetate, decylammonium acetate,
octadecylammonium acetate,
pyridiniumammonium acetate, cyclohexylammonium acetate, diethylammonium
acetate, propylethylammonium acetate, propyidiethylammonium acetate,
butylethylammonium acetate, methylhexylammonium acetate, tetramethylammonium
acetate, tetraethylammonium acetate, tetrapropylammonium acetate,
tetrabutylammonium acetate, dimethydiethylammonium acetate, triethylammonium
acetate, tripropylammonium acetate, tributylammonium acetate,
tetrapropylammonium acetate, tetrabutylammonium acetate, and mixtures of any
one or more of the above. The counterion agent includes an anion, e.g.,
acetate, carbonate, bicarbonate, phosphate, sulfate, nitrate, propionate,
formate, chloride, perchlorate, and bromide. However, the most preferred
counterion agent is triethylammonium acetate.

BSPE:
In the preferred embodiment, precautions are taken during the production of the
polymeric monolith so that it is substantially free of multivalent cation
contamination and the monolith is treated, for example, by an acid wash
treatment, to remove any residual surface metal contaminants. In one
embodiment, the monolith is characterized by having a DNA Separation Factor of
at least 0.05. In a preferred embodiment, the monolith is characterized by
having a DNA Separation Factor of at least 0.5. Also in a

preferred
embodiment, the **monolith** is characterized by having a Mutation Separation
Factor of at least 0.1.

BSPR:
In another aspect, the present invention is a method for treating the non-polar
surface of a polymeric medium used for separating polynucleotides, such as the
surface of beads in a MIPC column or the interstitial spaces in a polymeric
monolith, in order to improve the resolution of polynucleotides, such as dsDNA,
separated on said surface. This treatment includes contacting the surface with
a solution containing a multivalent cation binding agent. In a preferred
embodiment, the solution has a temperature of about 50.degree. C. to
90.degree. C. An example of this treatment includes flowing a solution
containing a multivalent cation binding agent through a MIPC column, wherein
the solution has a temperature of about 50.degree. C. to 90.degree. C. The
preferred temperature is about 70.degree. C. to 80.degree. C. In a preferred
embodiment, the multivalent cation binding agent is a coordination compound,
examples of which include water-soluble chelating agents and crown ethers.
Specific examples include acetylacetone, alizarin, aluminon, chloranilic acid,
kojic acid, morin, rhodizonic acid, thionalide, thiourea, .alpha.-furildioxime,
nioxime, salicylaldoxime, dimethylglyoxime, .alpha.-furildioxime, cupferron,
.alpha.-nitroso-.beta.-naphthol, nitroso-R-salt,
[illegible],
diphenylcarbazone, erichrome black I, PAN, PADA,
[illegible],
murexide, .alpha.-benzoinoxime, [illegible],
[illegible], glycine, triaminotriethylamine, thionalide,
triethylenetetramine, ethylenediaminetetraacetic acid (EDTA),
metalphthalein,
arsonic acids, .alpha.,.alpha.'-bipyridine,
4-hydroxybenzothiazole,
8-hydroxyquinaldine, 8-hydroxyquinoline, 1,10-phenanthroline,
picolinic acid,

quinaldic acid, .alpha.,.alpha.',.alpha."-terpyridyl, 9-methyl-2,3,7-trihydroxy-6-fluorone, pyrocatechol, salicylic acid, tiron, 4-chloro-1,2-dimercaptobenzene, dithiol, mercaptobenzothiazole, rubeanic acid, oxalic acid, sodium diethyldithiocarbarbamate, and zinc dibenzyldithiocarbamate. However, the most preferred chelating agent is EDTA.

In this aspect of the invention, the solution preferably includes an organic solvent as exemplified by alcohol, nitrile, dimethylformamide, tetrahydrofuran, ester, ether, and mixtures thereof. Examples of suitable solvents include methanol, ethanol, 2-propanol, 1-propanol, tetrahydrofuran, ethyl acetate, acetonitrile, and mixtures thereof. The most preferred organic solvent is acetonitrile. In one embodiment, the solution can include a counterion agent such as lower primary, secondary and tertiary amines, and lower trialkyammonium salts, or quaternary ammonium salts. More specifically, the counterion agent can be octylammonium acetate, octadimethylammonium acetate, decylammonium acetate, octadecylammonium acetate, pyridiniumammonium acetate, cyclohexylammonium acetate, diethylammonium acetate, propylethylammonium acetate, propyldiethylammonium acetate, butylethylammonium acetate, methylhexylammonium acetate, tetramethylammonium acetate, tetraethylammonium acetate, tetrapropylammonium acetate, tetrabutylammonium acetate, dimethydiethylammonium acetate, triethylammonium acetate, tripropylammonium acetate, tributylammonium acetate, tetrapropylammonium acetate, tetrabutylammonium acetate, and mixtures of any one or more of the above. The counterion agent includes an anion, e.g., acetate, carbonate, bicarbonate, phosphate, sulfate, nitrate, propionate, formate, chloride, perchlorate, and oxide. However, the most preferred counterion agent is triethylammonium acetate.

BRIEF:

In yet a further aspect, the invention provides a method for storing a medium used for separating polynucleotides, e.g., the beads of a MIPC

column or a
polymeric **monolith,** in order to improve the resolution of double stranded DNA
fragments separated using the medium. In the case of a MIPC column, the
preferred method includes flowing a solution containing a multivalent cation
binding agent through the column prior to storing the column. In a preferred
embodiment, the multivalent cation binding agent is a coordination compound,
examples of which include water-soluble chelating agents and crown ethers.
Specific examples include acetylacetone, alizarin, aluminon, chloranilic acid,
kojic acid, morin, rhodizonic acid, thionalide, thiourea, .alpha.-furildioxime,
nioxime, salicylaldoxime, dimethylglyoxime, .alpha.-furildioxime, cupferron,
.alpha.-nitroso-.beta.-naphthol, nitroso-R-salt, diphenylthiocarbazone,
diphenylcarbazone, eriochrome black T, PAN, SPADNS, glyoxal-bis(2-hydroxyanil),
murexide, .alpha.-benzoinoxime, mandelic acid, anthranilic acid, ethylenediamine, glycine, triaminotriethylamine, thionalide, triethylenetetramine, EDTA, metalphthalein, arsonic acids, .alpha.,.alpha.'-bipyridine, 4-hydroxybenzothiazole, 8-hydroxyquinaldine, 8-hydroxyquinoline, 1,10-phenanthroline, picolinic acid, quinaldic acid, .alpha.,.alpha.',.alpha."-terpyridyl, 9-methyl-2,3,7-trihydroxy-6-fluorone, pyrocatechol, salicylic acid, tiron, 4-chloro-1,2-dimercaptobenzene, dithiol, mercaptobenzothiazole, rubeanic acid, oxalic acid, sodium diethyldithiocarbamate, and zinc dibenzyldithiocarbamate. However, the most
preferred chelating agent is EDTA. In this aspect of the invention, the
solution preferably includes an organic solvent as exemplified by alcohols,
dioxane, dimethylformamide, tetrahydrofuran, esters, and others. The most
preferred organic solvent is acetonitrile. The solution can also include a
counterion agent such as lower primary, secondary and tertiary amines, and
lower trialkyammonium salts, or quaternary ammonium salts. More specifically,
the counterion agent can be octylammonium acetate,

octadimethylammonium
acetate, decylammonium acetate, octadecylammonium acetate,
pyridiniumammonium
acetate, cyclohexylammonium acetate, diethylammonium acetate,
propylethylammonium acetate, propyidiethylammonium acetate,
butylethylammonium
acetate, methylhexylammonium acetate, tetramethylammonium
acetate,
tetraethylammonium acetate, tetrapropylammonium acetate,
tetrabutylammonium
acetate, dimethydiethylammonium acetate, triethylammonium
acetate,
tripropylammonium acetate, tributylammonium acetate,
tetrapropylammonium

DEPR:
The medium can be enclosed in a column. In one embodiment, the
non-polar
surfaces comprise the surfaces of polymeric beads. In an
alternative
embodiment, the surfaces comprise the surfaces of interstitial
spaces in a
molded polymeric **monolith**. For purposes of simplifying the
description of the
invention and not by way of limitation, the separation of
polynucleotides using
nonporous beads, and the preparation of such beads, will be
primarily described
herein, it being understood that other separation surfaces, such
as the
interstitial surfaces of polymeric **monoliths**, are intended to be
included
within the scope of this invention. **Monoliths** such as rods
contain polymer
separation media which have been formed inside a column as a
unitary structure
having through pores or interstitial spaces which allow eluting
solvent and
analyte to pass through and which provide the non-polar
separation surfaces.

DEPR:
In another embodiment of the present invention, the
medium can be
the form of a polymeric **monolith** such as a rod-like **monolithic**
column. The
monolithic column is polymerized or formed as a single unit
inside of a tube as
described in the Examples hereinbelow. The through pore or
interstitial spaces

provide for the passage of eluting solvent and analyte materials. The separation is performed on the stationary surface. The surface can be porous, but is preferably nonporous. The form and function of the separations are identical to columns packed with beads. As with beads, the pores contained in the rod must be compatible with DNA and not trap the material. Also, the rod must not contain contamination that will trap DNA.

DEPR:
The molded polymeric rod of the present invention is prepared by bulk free radical polymerization within the confines of a chromatographic column. The base polymer of the rod can be produced from a variety of polymerizable monomers. For example, the **monolithic** rod can be made from polymers, including mono- and di-vinyl substituted aromatic compounds such as styrene, substituted styrenes, alpha-substituted styrenes and divinylbenzene; acrylates and methacrylates; polyolefins such as polypropylene and polyethylene; polyesters; polyurethanes; polyamides; polycarbonates; and substituted polymers including fluorosubstituted ethylenes commonly known under the trademark TEFLON. The base polymer can also be mixtures of polymers, non-limiting examples of which include poly(glycidyl methacrylate-co-ethylene dimethacrylate), poly(styrene-divinylbenzene) and poly(ethylvinylbenzene-divinylbenzene. The rod can be unsubsituted or substituted with a substituent such as a hydrocarbon alkyl or an aryl group. The alkyl group optionally has 1 to [illegible] carbon atoms in a straight or branched chain, and includes straight chain, branch chained, cyclic, saturated, unsaturated [illegible] functional groups of various types including aldehyde, ketone, ester, ether, alkyl groups, and the like, and the aryl groups includes as monocyclic, bicyclic, and tricyclic aromatic hydrocarbon groups including phenyl, naphthyl, and the like. In a

preferred embodiment, the alkyl group has 1-24 carbons. In a more preferred
embodiment, the alkyl group has 1-8 carbons. The substitution can also contain
hydroxy, cyano, nitro groups, or the like which are considered to be non-polar,
reverse phase functional groups. Methods for hydrocarbon substitution are
conventional and well-known in the art and are not an aspect of this invention.
The preparation of polymeric **monoliths** is by conventional methods well known in
the art as described in the following references: Wang et al. (J. Chromatog. A
699:230 (1994)), Petro et al. (Ana. Chem. 68:315 (1996)), and the following
U.S. Pat. Nos. 5,334,310; 5,453,185; 5,522,994 (to Frechet). **Monolith** or
rod columns are commercially available form Merck & Co (Darmstadt, Germany).

DEPR:
A chromatography tube in which the **monolith** polymeric separation medium is
prepared is made of stainless steel. The monomers, styrene (Sigma--Aldrich
Chemical Corp.) and divinylbenzene (Dow Chemical Corp.) are dried over
magnesium sulfate and distilled under vacuum.

DEPR:
Following polymerization, the rubber plugs are replaced by column end fittings
and the column is connected to an HPLC system. The HPLC instrument has a
low-pressure mixing quaternary gradient capability. A cartridge or guard
column containing an iminodiacetate multivalent cation capture resin is placed
in line between the column and the mobile phase source reservoir. The column
was washed by flowing 100 mL of tetrahydrofuran (THF) at 1 mL/min through
the column to remove the dodecyl alcohol and toluene, thereby creating
through-pores in the otherwise solid polymer **monolith**.

DEPR:
The non-polar, organic polymer **monolith** column is washed by flowing

tetrahydrofuran through the column at a flow rate of 2 mL per minute for 10
minutes followed by flowing methanol through the column at 2 mL per minute for
10 minutes. The non-polar, organic polymer **monolith** column is washed further
by flowing a mixture containing 100 mL of tetrahydrofuran and 100 mL of
concentrated hydrochloric acid through the column at 10 mL per minute for 20
minutes. Following this acid treatment, the non-polar, organic polymer
monolith column is washed by flowing tetrahydrofuran/water (1:1) through the
column at 2 mL per minute until neutral (pH 7).

DEPR:
Any double bonds remaining on the surface of the **monolith** column prepared in
Example 9 are reacted with bromine as described in Example 7.

DEPC:
Preparation of a Non-Polar Organic Polymer **Monolith** Chromatography Column

DEPC:
Bromination of Remaining Double Bonds on the Surface of Non-Polar Organic
Polymer **Monolith** Column

DEPC:
Nitration of a Non-Polar Organic Polymer **Monolith** Column

CCXF:
210/198.2

OREF:
Nakanishi et al. Double Pore Silica Gel **Monolith** Applied to Liquid
[illegible] Science & Technology, vol. 8, pp. [illegible], 1997.

OREF:
Petro et al, Molded **Monolithid** Bed of Macrophrous Poly(Styrene-Co-Divinylbenzene) as a Separation Medium for PHLC of Synthetic
Polymers . . . , Analytical Chemistry, 68:315-321 (1996).

DOCUMENT-IDENTIFIER: US 6056877 A
TITLE: Non-polar media for polynucleotide separations

BSPR:
The present invention is directed to the separation of polynucleotides using a separation medium having non-polar surfaces, such as the surfaces of nonporous beads or surfaces of interstitial spaces within a molded **monolith** (e.g., a derivatized silica **monolith**), which surfaces are substantially free from contamination with multivalent cations. More specifically, the invention is directed to the chromatographic separation of both single stranded and double stranded polynucleotides by chromatography using a nonporous separation medium, where the medium is either organic or inorganic material which is coated with a polymer, or non-polar substituted polymer, and/or which has substantially all surface substrate groups substituted with a non-polar hydrocarbon or non-ionic substituted hydrocarbon.

BSPR:
These and other objects of the invention, which will become apparent from reading the following specification, have been achieved by the method of the present invention in which polynucleotides are separated using a nonporous separation medium such as beads or a molded **monolith** (e.g., a silica gel **monolith**), where the medium comprises either organic or inorganic material which is coated with a polymer, or non-polar substituted polymer, and/or which has substantially all surface substrate groups substituted with a non-polar hydrocarbon or non-ionic substituted hydrocarbon.

BSPR:
In one aspect, the invention is a method for separating a mixture of polynucleotides comprising applying a mixture of polynucleotides

having up to
1500 base pairs to a separation medium, the separation surfaces of the medium
coated with a hydrocarbon or non-polar hydrocarbon substituted polymer, or
having substantially all polar groups reacted with a non-polar hydrocarbon or
substituted hydrocarbon group, wherein said surfaces are non-polar; and eluting
the polynucleotides. The separation medium can be enclosed in a column.
Examples of non-polar surfaces include the surfaces of beads such as nonporous
particles and the surfaces of intersitital spaces within a **monolith** (e.g., a
silica gel **monolith**), which surfaces are coated with a hydrocarbon or non-polar
substituted polymer or having substantially all surface substrate groups
reacted with a non-polar hydrocarbon or substituted hydrocarbon group. In the
preferred embodiment, precautions are taken during the production of the medium
so that it is substantially free of multivalent cation contaminants and the
medium is treated, for example by an acid wash treatment and/or treatment with
multivalent cation binding agent, to substantially remove any residual surface
metal contaminants. The preferred separation medium is characterized by having
a DNA Separation Factor (defined hereinbelow) of at least 0.05. The preferred
medium is characterized by having a Mutation Separation Factor (as defined
hereinbelow) of at least 0.1. In a preferred embodiment, the separation is
made by Matched Ion Polynucleotide Chromatography (MIPC, as defined
[illegible]. The elution step preferably uses a mobile phase containing a
[illegible] water-soluble organic solvent. Examples of a suitable
organic solvent include alcohol, nitrile, dimethylformamide, tetrahydrofuran,
ester, ether, and mixtures of two or more thereof, e.g., methanol, ethanol,
2-propanol, 1-propanol, tetranydrofuran, ethyl acetate, acetonitrile. The most
preferred organic solvent is acetonitrile. The counterion agent

is preferably
selected from the group consisting of lower primary amine, lower secondary
amine, lower tertiary amine, lower trialkyammonium salt, quaternary ammonium
salt, and mixtures of one or more thereof. Non-limiting examples of counterion
agents include octylammonium acetate, octyidimethylammonium acetate,
decylammonium acetate, octadecylammonium acetate,
pyridiniumammonium acetate,
cyclohexylammonium acetate, diethylammonium acetate, propylethylammonium
acetate, propyldiethylammonium acetate, butylethylammonium acetate,
methylhexylammonium acetate, tetramethylammonium acetate, tetraethylammonium
acetate, tetrapropylammonium acetate, tetrabutylammonium acetate,
dimethydiethylammonium acetate, triethylammonium acetate, tripropylammonium
acetate, tributylammonium acetate, and mixtures of any one or more of the
above. The counterion agent includes an anion, e.g., acetate, carbonate,
bicarbonate, phosphate, sulfate, nitrate, propionate, formate, chloride,
perchlorate, or bromide. The most preferred counterion agent is triethylammonium acetate or triethylammonium hexafluoroisopropyl alcohol.

BSPR:
In a still further aspect, the invention is a method for separating a mixture
of polynucleotides comprising applying a mixture of polynucleotides having up
to 1500 base pairs to a **monolith** having non-polar separation surfaces, and
eluting the polynucleotides. The **monolith** can be enclosed in a column or other
[illegible] system, such as a cartridge. In a preferred embodiment, the
monolith [illegible] **monolith**. The non-polar separation surfaces include
the surfaces of interstitial spaces within the **monolith,** which surfaces are
coated with a hydrocarbon or non-polar substituted polymer or having
substantially all surface substrate groups reacted with a non-polar hydrocarbon
or substituted hydrocarbon group. An example of a suitable

monolith is one which is polyfunctionally derivatized with octadecylsilyl groups. In the preferred embodiment, precautions are taken during the production of the **monolith** so that it is substantially free of multivalent cation contaminants and the **monolith** is treated, for example by an acid wash treatment and/or treatment with multivalent cation binding agent, to substantially remove any residual surface metal contaminants. The preferred **monolith** is characterized by having a DNA Separation Factor of at least 0.05. The preferred **monolith** is characterized by having a Mutation Separation Factor of at least 0.1. In a preferred embodiment, the separation is made by Matched Ion Polynucleotide Chromatography. The elution step preferably uses a mobile phase containing a counterion agent and a water-soluble organic solvent. Examples of a suitable organic solvent include alcohol, nitrile, dimethylformamide, tetrahydrofuran, ester, ether, and mixtures of one or more thereof, e.g., methanol, ethanol, 2-propanol, 1-propanol, tetrahydrofuran, ethyl acetate, acetonitrile. The most preferred organic solvent is acetonitrile. The counterion agent is preferably selected from the group consisting of lower primary amine, lower secondary amine, lower tertiary amine, lower trialkyammonium salt, quaternary ammonium salt, and mixtures of one or more thereof. Non-limiting examples of counterion agents include octylammonium acetate, octyldimethylammonium acetate, [illegible] acetate, hexadecylammonium acetate, pyridinium acetate, [illegible] acetate, diethylammonium acetate, propylethylammonium acetate, propyldiethylammonium acetate, butylethylammonium acetate, methylhexylammonium acetate, tetramethylammonium acetate, tetraethylammonium acetate, tetrapropylammonium acetate, tetrabutylammonium acetate, dimethydiethylammonium acetate, triethylammonium acetate, tripropylammonium

acetate, tributylammonium acetate, and mixtures of any one or more of the
above. The counterion agent includes an anion, e.g., acetate, carbonate,
bicarbonate, phosphate, sulfate, nitrate, propionate, formate, chloride,
perchlorate, or bromide, The most preferred counterion agent is triethylammonium acetate or triethylammonium hexafluoroisopropyl alcohol.

BSPR:
In a yet further aspect, the invention provides a **monolith** having non-polar
separation surfaces which are substantially free from contamination with
multivalent cations. The **monolith** can be enclosed in a column or other
containment system, such as a cartridge. The non-polar separation surfaces
include the surfaces of interstitial spaces within the **monolith** (e.g., a silica
monolith), which surfaces are coated with a hydrocarbon or non-polar
substituted polymer or having substantially all surface substrate groups
reacted with a non-polar hydrocarbon or substituted hydrocarbon group. An
example of a suitable **monolith** is one which is derivatized with
polyfunctionally derivatized octadecylsilyl groups. In the preferred
embodiment, precautions are taken during the production of the **monolith** so that
it is substantially free of multivalent cation contaminants and the **monolith** is
treated, for example by an acid wash treatment and/or treatment with
multivalent cation binding agent, to remove any residual surface metal
contaminants. The preferred **monolith** is characterized by having a DNA
Separation Factor of at least 0.05. The preferred **monolith** is [illegible]
having a Mutation Separation Factor of at least [illegible].

BSPR:
In another aspect, the present invention is a method for treating the non-polar
surfaces of a medium used for separating polynucleotides, such as the surfaces
of beads in a MIPC column or the surfaces of interstitial spaces

in a **monolith,**
in order to improve the resolution of polynucleotides, such as dsDNA, separated
on said surfaces. This treatment includes contacting the surface with a
solution containing a multivalent cation binding agent. In a preferred
embodiment, the solution has a temperature of about 50.degree. C. to
90.degree. C. An example of this treatment includes flowing a solution
containing a multivalent cation binding agent through a MIPC column, wherein
the solution has a temperature of about 50.degree. C. to 90.degree. C. The
preferred temperature is about 70.degree. C. to 80.degree. C. In a preferred
embodiment, the multivalent cation binding agent is a coordination compound,
examples of which include water-soluble chelating agents and crown ethers.
Specific examples include acetylacetone, alizarin, aluminon, chloranilic acid,
[illegible] acid, morin, rhodizonic acid, thionalide, thiourea, .alpha.-furildioxime,
nioxime, salicylaldoxime, dimethylglyoxime, .alpha.-furildioxime, cupferron,
.alpha.-nitroso-.beta.-naphthol, nitroso-R-salt, diphenylthiocarbazone,
diphenylcarbazone, eriochrome black T, PAN, SPADNS, glyoxal-bis(2-hydroxyanil),
murexide, .alpha.-benzoinoxime, mandelic acid, anthranilic acid,
ethylenediamine, glycine, triaminotriethylamine, thionalide, triethylenetetramine, ethylenediaminetetraacetic acid (EDTA), metalphthalein,
arsonic acids, .alpha., .alpha.'-bipyridine, 4-hydroxybenzothiazole,
8-hydroxyquinaldine, 8-hydroxyquinoline, 1,10-phenanthroline, picolinic acid,
[illegible] acid, .alpha...alpha.',.alpha."-terpyridyl,
6-methyl-2,3,7-trihydroxy-6-fluorone, pyrocatechol, salicylic acid, [illegible],
4-chloro-1,2-dimercaptobenzene, dithizone, [illegible], tannic acid,
oxalic acid, sodium diethyldithiocarbamate, and zinc dibenzyldithiocarbamate. However, the most preferred chelating agent is EDTA.
In this aspect of the invention, the solution preferably includes an organic
solvent as exemplified by alcohol, nitrile, dimethylformamide,

tetrahydrofuran,
ester, ether, and mixtures thereof. Examples of suitable

BSPR:
In yet a further aspect, the invention provides a method for storing a medium
used for separating polynucleotides, e.g., the beads of a MIPC column or a
monolith, in order to improve the resolution of double stranded DNA fragments
separated using the medium. In the case of a MIPC column, the preferred method
includes flowing a solution containing a multivalent cation binding agent
through the column prior to storing the column. In a preferred embodiment, the
multivalent cation binding agent is a coordination compound, examples of which
include water-soluble chelating agents and crown ethers. Specific examples
include acetylacetone, alizarin, aluminon, chloranilic acid, kojic acid, morin,
rhodizonic acid, thionalide, thiourea, (.alpha.-furildioxime, nioxime,
salicylaldoxime, dimethylglyoxime, .alpha.-furildioxime, cupferron,
.alpha.-nitroso-.beta.-naphthol, nitroso-R-salt, diphenylthiocarbazone,
diphenylcarbazone, eriochrome black T, PAN, SPADNS, glyoxal-bis(2-hydroxyanil),
murexide, .alpha.-benzoinoxime, mandelic acid, anthranilic acid,
ethylenediamine, glycine, triaminotriethylamine, thionalide, triethylenetetramine, EDTA, metalphthalein, arsonic acids, .alpha.,.alpha.'-bipyridine, 4-hydroxybenzothiazole, 6-hydroxyquinaldine,
6-hydroxyquinoline, 1,10-phenanthroline, picolinic acid, quinaldic acid,
.alpha.,.alpha.',.alpha."-terpyridyl,
9-methyl-2,3,7-trihydroxy-6-fluorone,
[illegible] salicylic acid, tiron,
4-[illegible]-1,2-dimercaptobenzene, dithiol,
[illegible], mimosic acid, oxalic acid, sodium diethyldithiocarbamate, and zinc diethyl dithiocarbamate. However, the most
preferred chelating agent is EDTA. In this aspect of the invention, the
solution preferably includes an organic solvent as exemplified by alcohols,
nitriles, dimethylformamide, tetrahydrofuran, esters, and ethers. The most

preferred organic solvent is acetonitrile. The solution can also include a
counterion agent such as lower primary, secondary and tertiary amines, and
lower trialkyammonium salts, or quaternary ammonium salts. More specifically,
the counterion agent can be octylammonium acetate, octadimethylammonium
acetate, decylammonium acetate, octadecylammonium acetate, pyridiniumammonium
acetate, cyclohexylammonium acetate, diethylammonium acetate, propylethylammonium acetate, propyldiethylammonium acetate, butylethylammonium
acetate, methylhexylammonium acetate, tetramethylammonium acetate,
tetraethylammonium acetate, tetrapropylammonium acetate, tetrabutylammonium
acetate, dimethydiethylammonium acetate, triethylammonium acetate,
tripropylammonium acetate, tributylammonium acetate, tetraethylammonium
acetate, tetrapropylammonium acetate, tetrabutylammonium acetate, and mixtures
of any one or more of the above. The counterion agent includes an anion, e.g.,
acetate, carbonate, bicarbonate, phosphate, sulfate, nitrate, propionate,
formate, chloride, perchlorate, and bromide. However, the most preferred
counterion agent is triethylammonium acetate.

DEPR:
The medium can be enclosed in a column. In one embodiment, the non-polar
surfaces comprise the surfaces of beads. In an alternative embodiment, the
surfaces comprise the surfaces of interstitial spaces in a molded monolith.
For purposes of simplifying the description of the invention and [illegible]
limitation, the separation of polynucleotides using [illegible]
preparation of such beads, will be [illegible], it being
understood that other separation surfaces, such as the interstitial surfaces of
monoliths, are intended to be included within the scope of this invention.
Monoliths such as derivatized silica gel rods contain separation media which

have been formed inside a column as a unitary structure having through pores or
interstitial spaces which allow eluting solvent and analyte to pass through and
which provide the non-polar separation surface.

DEPR:
In another embodiment of the present invention, the separation medium can be in
the form of a **monolith** such as a rod-like **monolithic** column. The **monolithic**
column can be polymerized or formed as a single unit inside of a tube. The
through pore or interstitial spaces provide for the passage of eluting solvent
and analyte materials. The separation is performed on the stationary surface.
The surface can be porous, but is preferably nonporous. The form and function
of the separations are identical to columns packed with beads. As with beads,
the pores contained in the rod must be compatible with DNA and not trap the
material. Also, the rod must not contain contamination that will trap DNA.

DEPR:
In one embodiment of the present invention, the separation medium is continuous
monolithic silica gel. A molded **monolith** can be prepared by polymerization
within the confines of a chromatographic column (e.g., to form a rod) or other
containment system. A **monolith** is preferably obtained by the hydrolysis and
polycondensation of alkoxysilanes. A preferred **monolith** is derivatized in
order to produce non-polar interstitial surfaces. Chemical modification of
silica **monoliths** with octadecyl, methyl or other ligands can be carried out.
A preferred derivatized **monolith** is one which is polyfunctionally
derivatized with octadecylsilyl groups The preparation of derivatized silica
monoliths is by conventional methods well known in the art as described in
Example 15 and in the following references which are hereby incorporated in
their entirety herein: Nakanishi, et al., J. Sol-Gel Sci.

Technol. 8:547
(1997); Nakanishi, et al., Bull, Chem. Soc. Jpn. 67:1327
(1994); Cabrera, et
al., Trends Analytical Chem, 17:50 (1998); Jinno, et al.,
Chromatographia
27:288 (1989).

DEPR:
The non-polar, derivatized silica **monolith** column is washed by flowing
tetrahydrofuran through the column at a flow rate of 2 mL per minute for 10
minutes followed by flowing methanol through the column at 2 mL per minute for
10 minutes. The non-polar **monolith** column is washed further by flowing a
mixture containing 100 mL of tetrahydrofuran and 100 mL of concentrated
hydrochloric acid through the column at 10 mL per minute for 20 minutes.
Following this acid treatment, the **monolith** column is washed by flowing
tetrahydrofuran/water (1:1) through the column at 2 mL per minute until neutral
(pH 7).

DEPC:
Preparation of a Silica **Monolith**

CCNR:
210/198.2

ORPL:
Nakanishi et al. Double Pore Silica Gel **Monolith** Applied to Liquid
Chromatography, J. Sol-Gel Science & Technology, vol. 8, pp. 547-552, 1997.

ORPL:
Peters et al, Molded **Monolithid** Bed of Macroporous
Poly(Styrene-Co-Divinylbenzene) as a Separation Medium for PHLC
[illegible]
Polymers . . . , Analytical Chemistry, 68: [illegible],.

DOCUMENT-IDENTIFIER: US 5779891 A
TITLE: Non-fouling flow through capacitor system

BSPR:
The electrodes may be made out of any **monolithic** high surface area conductive materials, in at least one anode/cathode pair. Where the high surface area material is conductive, but not optimally conductive, an electrical conductive backing may be employed. High surface area conductive materials suitable for use in the present invention include, but are not limited to: activated carbon; activated carbon treated with a halogen; carbon foams; carbon aerogel and aerogel composite materials; nanotubes; conductive polymers, especially in porous or network form; polymerized fullerenes; or any high surface area conductive material may be used. Conductive ceramics may also be used, either by themselves or impregnated onto high surface area substrates, including various forms of carbon such as fiber, foam, powder or aerogel. In general, absorbing any electrically actuated small or large molecule onto the conductive high surface area material that improves the capacitance will improve the function of the capacitor. Another preferred high surface area conductive material is conductive transition metal oxides, nitrides, or borides prepared using sol/gel technique. Powdered high surface area materials may be sintered to **monolithic** electrodes or bound together with binder materials.

BSPR:
Additionally, conducting electrodes where no backing layer is required would include high surface area preparations of graphitic carbon, high surface area expanded metals, metal fibers, or metal meshes. For example, titanium fibers coated with high surface area platinum series black are known and

are marketed
as electrode materials. Other examples include platinum coated niobium and
foamed metals. High surface area carbon materials may be mixed with metal or
graphitic fibers or meshes and formed into **monolithic** units.

DRPR:
FIGS. 13A-F are schematic illustrations of various **monolithic** electrodes for
use in the capacitor of the invention;

DEPR:
FIGS. 13A-F depict various **monolithic** electrode designs that incorporate an
inner conductive backing. This is useful for all the above flow-through
capacitors because a compression fitting is no longer required to make a
contact between the high surface area layers and the conductive backing layers.
The electrodes of FIG. 13 contain an inner conductive backing layer 2, which
may be a metal foil, graphite foil, a fibrous material, or an interpenetrating
network mesh material. In foil form, this backing material has many holes 35
therethrough to allow communication and interconnection with the high surface
area material that forms a sandwich on both sides in a flat electrode.
Alternatively, a rod style conductor can be used, with the high surface area
material 1 formed directly around a central rod or wire conductor 32. This
material is bonded together or calcined as a single, **monolithic** piece,
containing the conductive backing internally. For example, activated carbon or
[illegible] may be mixed with a phenolic binder and hot pressed to form the
[illegible] in FIG. 13, prior to calcining in the absence of air. The
interconnections formed through the holes in the [illegible] backing hold the
high surface area material together and prevent it from pulling away from the
backing due to shrinkage during calcining. Alternatively, carbon films or
layers may be deposited onto conductive backings, and activated in place.

Integral leads 4 are formed from the internal conductive backing layer or rod
38.

CLPF:
20. The system of claim 1 wherein the **monolithic** high surface area material is
selected from the group consisting of: bonded or sintered activated carbon
particles; aerogel particles; conductive ceramics; activated carbon fiber
cloth; fibrous metal coated with platinum; or transition metal oxides,
borides and nitrides, and combinations thereof.

CLPF:
21. The system of claim 1 wherein the **monolithic** high surface area material
comprises activated carbon sintered together with a binder and doped with a
metal.

CLPV:
A [illegible] or more fouling-resistant, flow-through capacitors, each capacitor having
at least one anode and cathode pair for use in the electrical purification,
concentration, separation, recovery, or electrochemical breakdown of solutes or
fluids, which capacitor comprises one or more **monolithic,** spaced apart pairs of
cathode-anode electrodes incorporating a high surface area material and having
a non-conductive spacer between the anode and cathode electrodes characterized
by an open flow path between the electrodes to permit the unobstructed flow of
the fluid across the electrode surface and of sufficient width to prevent the
[illegible] of the capacitor, and wherein the open flow path has at least one
[illegible] exterior of the capacitor; and

EXPR:
210/198.2

DOCUMENT-IDENTIFIER: US 5772875 A
TITLE: Chromatography column

ABPR:
A liquid chromatography column including a chromatographic matrix, liquid flow inlet means and liquid flow outlet means, and a distributor located adjacent the inlet and functioning to distribute the incoming liquid. The column includes a matrix is that is **monolithic** and porous; and in that when eluant passes through the matrix a liquid accommodating gap which is devoid of matrix material is present between the matrix and the distributor.

BSPR:
An alternative to matrix beds which consist in packed particles is found in the so-called continuous matrices (also called **monolithic** matrices) which have been porous. This type of matrix does not tend to form channels as a result of subsidence or settling of the bed. By continuous (**monolithic**) matrices is meant matrices which are intrinsically coherent. Matrices which consist of packed membranes or filters are not **monolithic**.

BSPR:
The inventive liquid chromatography column is constructed from a column tube which includes a chromatography matrix and a distributor or spreader placed adjacent the inlet and functioning to distribute the incoming liquid. The column is characterized in that the matrix is **monolithic** and porous and in that [illegible] through the matrix, there is present between the matrix and the distributor a gap which does not contain matrix material. The remainder of the column may be of known design. The width of the gap is such as to impart improved properties to the column with respect to number of theoretical plates, symmetry factors and elution volumes in

comparison with the
case when the distributor plate abuts the matrix.

BSPR:
No gap is required in zero liquid flow conditions, but can arise as a result of
compression of the **monolithic** matrix by the liquid flow. In practice, it is
preferable that the gap is created when producing the column, when the inlet
adaptor and associated distributor or spreader is moved towards the matrix
inlet area. In this regard, we have found it very suitable to adapt the width
of the gap so that it is always discernible to the eye.

DEPR:
Monolithic matrices can be produced in different ways, for instance by the
polymerization of inverse emulsions where the oil phase includes polymerizable
monomers, or by bulk-polymerization together with a so-called porogen (a
solvent which can be washed out after polymerization).

DEPR:
The inventive column may include a number of **monolithic** chromatographic
matrices stacked one upon the other.

CLPR:
1. A liquid chromatography column, comprising a column tube including a
chromatographic matrix, the matrix being **monolithic** and porous, liquid flow
inlet means and liquid flow outlet means, a distributor located adjacent the
inlet for distributing incoming liquid, and, when eluant passes through the
matrix, a [illegible] accommodating gap which is devoid of matrix material, wherein
[illegible] accommodating gap is between the matrix and the distributor and is
directly adjacent the matrix, the gap being effective to improve a theoretical
plate number, an elution volume or a symmetry factor of the liquid
chromatography column.

CCOR:

DOCUMENT-IDENTIFIER: US 5766460 A
TITLE: Liquid chromatographic system

DEPR:
FIGS. 5a and 5b illustrate separation modules which include a separation medium in the form of a matrix (32) and flow channels. The module illustrated in FIG. 5a has an inlet and an outlet through a common end-connecting means, and the module illustrated in FIG. 5b through separate ends. The end pieces of the separation medium have spreading and collecting functions respectively, which can be achieved with the aid of filter paper inserts, end-piece abutment surfaces with inlets combined with systems of channels, etc. (33 and 34 in FIG. 4a). The matrix (32) may be comprised of discrete, packed particles of cross-linked polysaccharide, polyacrylamide, and the like, or may be continuous (**monolithic**), i.e. have the form of a porous body. The matrix may exhibit substituents enabling the desired type of chromatography to be run (e.g. ion exchange groups, hydrophobic groups, affinity ligands). The width and the length of the matrix are determined by the separation performance desired. The matrix may be given the form of a membrane. It is believed that the future preferred embodiments of the invention will comprise matrixes in form of a continuous body with a cylindrical or frusto conical shape, narrowing in the flow direction of the column (see FIGS. 4a-b).

CCOR:
210/198.2

DOCUMENT-IDENTIFIER: US 5728457 A
TITLE: Porous polymeric material with gradients

BSPR:
Because the plug columns are essentially a single molded polymer **monolith** traversed by large channels and permeated by small pores, their hydrodynamic properties are excellent. They are unlike any existing separation medium based on packed polymer beads because flow through the plug column does not involve any interstitial space but results entirely from the existence of the through channels built into the porous polymer **monolith**. Therefore, high rates of mass transfer can be used. Despite the high flow rates and steep gradients, separations using these plug columns are remarkably effective. The continuous polymer plug media afford excellent resolution in the separation of proteins, peptides and small molecules.

CCXR:
210/198.2

DOCUMENT-IDENTIFIER: US 5707589 A
TITLE: Funnel-shaped sample-vial septum with membrane covered diffusion-barrier section

ABPL:
A funnel-shaped **monolithic** low-density polyethylene sample-vial septum comprises a flange, a capture wall, a diffusion-barrier wall, and a membrane. The capture wall is conical, extending from its truncated apex at the diffusion barrier wall to its mouth about which the flange is disposed. The membrane is disposed at the end of the diffusion-barrier wall away from the capture wall. This structure defines a septum aperture, including a conical capture section, defined by the capture wall, and a cylindrical diffusion-barrier section, defined by the diffusion-barrier wall. The 7.0 mm length of the diffusion-barrier section is 12/mm times the square of its diameter 0.76 mm. The diameter of the diffusion-barrier section which is selected to be 0.05 mm greater than the diameter of the largest needle to be used with the septum, i.e., a 22 gauge needle typically used for liquid chromatography. The minimum thickness of the membrane is 0.05 mm so that a blunt 26 gauge needle will not be damaged while piercing the membrane. The membrane has a curved surface facing the capture section. The radius of curvature of this surface is 7.0 mm, set equal to the barrier section length. This is a result of a [illegible] tip that is curved so that if misaligned, the desired minimum [illegible] thickness is still achieved. [illegible] the membrane serves as a [illegible] barrier to evaporative sample loss. After piercing, the [illegible] diffusion-barrier section serves as an effective barrier to evaporative sample loss.

BSPR:

The septum is preferably **monolithic** and preferably formed of polymer. By "**monolithic**" is meant that it is fabricated from a single piece of material, as by molding or machining, rather than formed by assembling or fusing separate components. The **monolithic** structure includes not only the walls of the aperture, but the flange and the membrane as well.

DEPR:
In accordance with the present invention, a **monolithic** funnel-shaped sample-vial septum A1 comprises a flange 12, a capture wall 14, a diffusion barrier wall 16, and a membrane 18, as shown in FIG. 1. Collectively, these elements define a septum aperture 20 comprising a capture section 22, defined by capture wall 14, and a diffusion-barrier section 24, defined by diffusion barrier wall 16. Capture wall 14 is generally conical with its apex end 30 truncated where it merges with one end 32 of cylindrical diffusion barrier wall 16. The other end 34 of the diffusion barrier wall 16 is sealed by membrane 18, as shown in FIG. 2. The wide end 36 of capture wall 14 is ringed by annular flange 12.

CCXR:
210/198.2

DOCUMENT-IDENTIFIER: US 5653875 A
TITLE: Nucleophilic bodies bonded to siloxane and use thereof for separations
from sample matrices

BSPR:
The invention further contemplates a structure comprising discrete adsorbent
bodies bonded to a **monolithic** substrate through a medium comprising a siloxane
polymer.

DEPR:
Where the invention is embodied in a chromatographic apparatus or solid phase
adsorption device, discrete adsorbent bodies are typically bonded to a
monolithic or essentially **monolithic** substrate, such as an interior wall of a
chromatographic column, or a fiber or contiguous network of fibers that support
the adsorbent particles of a solid phase adsorption device. As used herein,
the term "**monolithic**" includes essentially **monolithic** structures such as a
weave or mat of contiguous fibers.

DEPR:
In certain applications known to the art, a particulate substrate may provide
desirable functions or advantages. It will be understood that, in certain
embodiments, the novel chromatographic apparatus or solid phase adsorption
device of the invention may comprise a substrate which itself comprises
[illegible], to which discrete adsorbent bodies are bonded with a siloxane
[illegible]. There may also be applications other than in chromatography, sample
preparation, or catalysis wherein discrete bodies having a functional surface
property are bonded to a **monolithic** or particulate substrate via the medium of
a siloxane polymer.

CCOR:

DOCUMENT-IDENTIFIER: US 5647979 A
TITLE: One-step preparation of separation media for reversed-phase chromatography

BSPR:
These disadvantages are eliminated by the formation of a continuous bed in the capillary, i.e., a **monolithic** porous polymer used in place of the beads, the polymer having been formed by polymerization in the capillary itself, spanning the entire cross section of the capillary and bonded to the capillary wall. A description of this type of bed is found in granted European Patent Specification No. 0 407 560 of Bio-Rad Laboratories, Inc., and its United States counterpart, pending application Ser. No. 08/400,419, filed Mar. 2, 1995. The disclosures of both of these documents are incorporated herein by reference.

CCOR:
210/198.2

DOCUMENT-IDENTIFIER: US 5630937 A
TITLE: Nucleophilic bodies bonded to siloxane and use thereof for separations
from sample matrices

BSPR:
The invention further contemplates a structure comprising discrete adsorbent
bodies bonded to a **monolithic** substrate through a medium comprising a siloxane
polymer.

DEPR:
Where the invention is embodied in a chromatographic apparatus or solid phase
adsorption device, discrete adsorbent bodies are typically bonded to a
monolithic or essentially **monolithic** substrate, such as an interior wall of a
chromatographic column, or a fiber or contiguous network of fibers that support
the adsorbent particles of a solid phase adsorption device. As used herein,
the term "**monolithic**" includes essentially **monolithic** structures such as a
weave or mat of contiguous fibers.

DEPR:
In certain applications known to the art, a particulate substrate may provide
desirable functions or advantages. It will be understood that, in certain
embodiments, the novel chromatographic apparatus or solid phase adsorption
device of the invention my comprise a substrate which itself comprises discrete
bodies, to which discrete adsorbent bodies are bonded with a siloxane polymer.
There may also be applications other than in chromatography, such as
preparation, or catalysis wherein discrete bodies having a functional surface
property are bonded to a **monolithic** or particulate substrate via the medium of
a siloxane polymer.

CLPR:

1. A solid phase adsorption device comprising a **monolithic** substrate having
adsorbent bodies bonded to the surfaces thereof through a medium comprising a
siloxane polymer.

CCOR:
210/198.2

DOCUMENT-IDENTIFIER: US 5620603 A
TITLE: Nucleophilic bodies bonded to siloxane and use thereof for separations
from sample matrices

BSPR:
The invention further contemplates a structure comprising discrete adsorbent
bodies bonded to a **monolithic** substrate through a medium comprising a siloxane
polymer.

DEPR:
Where the invention is embodied in a chromatographic apparatus or solid phase
adsorption device, discrete adsorbent bodies are typically bonded to a
monolithic or essentially **monolithic** substrate, such as an interior wall of a
chromatographic column, or a fiber or contiguous network of fibers that support
the adsorbent particles or a solid phase adsorption device. As used herein,
the term "**monolithic**" includes essentially **monolithic** structures such as a
weave or mat of contiguous fibers.

DEPR:
In certain applications known to the art, a particulate substrate may provide
desirable functions or advantages. It will be understood that, in certain
embodiments, the novel chromatographic apparatus or solid phase adsorption
device of the invention may comprise a substrate which itself comprises
discrete bodies, to which discrete adsorbent bodies are bonded with a siloxane
polymer. There may also be applications other than in chromatography, sample
preparation, or catalysis wherein discrete bodies having a functional surface
property are bonded to a **monolithic** or particulate substrate via the medium of
a siloxane polymer.

CCXR:

DOCUMENT-IDENTIFIER: US 5620597 A
TITLE: Non-fouling flow-through capacitor

BSPR:
The electrodes may be made out of any **monolithic** high surface area conductive materials, in at least one anode/cathode pair. Where the high surface area material is conductive, but not optimally conductive, an electrical conductive backing may be employed. High surface area conductive materials suitable for use in the present invention include, but are not limited to: activated carbon; activated carbon treated with a halogen; carbon foams; carbon aerogel and aerogel composite materials; nanotubes; conductive polymers, especially in porous or network form; polymerized fullerenes; or any high surface area conductive material may be used. Conductive ceramics may also be used, either by themselves or impregnated onto high surface area substrates, including various forms of carbon such as fiber, foam, powder or aerogel. In general, absorbing any electrically actuated small or large molecule onto the conductive high surface area material that improves the capacitance will improve the function of the capacitor. Another preferred high surface area conductive material is conductive transition metal oxides, nitrides, or borides prepared using sol/gel technique. Powdered high surface area materials may be sintered into **monolithic** electrodes or bound together with binder materials.

DETR:
Intrinsically conductive electrodes where no backing layer is required would include high surface area preparations of graphitic carbon, high surface area expanded metals, metal fibers, or metal meshes. For example, titanium fibers coated with high surface area platinum series black are known and

are marketed as electrode materials. Other examples include platinum coated niobium and foamed metals. High surface area carbon materials may be mixed with metal or graphitic fibers or meshes and formed into **monolithic** units.

DRPR:
FIGS. 13A-F are schematic illustrations of various **monolithic** electrodes for use in the capacitor of the invention;

DEPR:
FIGS. 13A-F depict various **monolithic** electrode designs that incorporate an inner conductive backing. This is useful for all the above flow-through capacitors because a compression fitting is no longer required to make a contact between the high surface area layers and the conductive backing layers.
The electrodes of FIG. 13 contain an inner conductive backing layer 2, which may be a metal foil, graphite foil, a fibrous material, or an interpenetrating network mesh material. In foil form, this backing material has many holes 35 therethrough to allow communication and interconnection with the high surface area material that forms a sandwich on both sides in a flat electrode.
Alternatively, a rod style conductor can be used, with the high surface area material 1 formed directly around a central rod or wire conductor 32. This material is bonded together or calcined as a single, **monolithic** piece, containing the conductive backing internally. For example, activated carbon or aerogel powder may be mixed with a phenolic binder and hot pressed to form the shape in FIG. 13, prior to calcining in the absence of air. The interconnections formed through the holes in the conductive backing hold the high surface area material together and prevent it from pulling away from the backing due to shrinkage during calcining. Alternatively, carbon films or layers may be deposited onto conductive backings, and activated in place.

Integral leads 4 are formed from the internal conductive backing layer or rod
32.

CLPR:
1. A foul-resistant, flow-through capacitor having at least one anode and
cathode pair for use in the electrical purification, concentration, separation,
recovery, or electrochemical breakdown of solutes or fluids, which capacitor
comprises one or more **monolithic,** spaced apart pairs of cathode-anode
electrodes incorporating a high surface area material and having a
non-conductive spacer between the anode and cathode electrodes characterized by
an open flow path between the electrodes to permit the unobstructed flow of the
fluid across the electrode surface and of sufficient width to prevent the
fouling of the capacitor and wherein the open flow path has at least one
dimension open to an exterior of the capacitor.

CLPR:
13. The capacitor of claim 1 wherein the **monolithic** high surface area material
is selected from the group consisting of: bonded or sintered activated carbon
particles; aerogel particles; conductive ceramics; activated carbon fiber
cloth; fibrous metal coated with platinum; or transition metal oxides,
borides and nitrides and combinations thereof.

CLPR:
14. The capacitor of claim 1 wherein the **monolithic** high surface area material
comprises activated carbon sintered together with a binder and doped with a
metal.

[illegible]:
210/198.2

DOCUMENT-IDENTIFIER: US 5609756 A
TITLE: Nucleophilic bodies bonded to siloxane and use thereof for separations
from sample matrices

BSPR:
The invention further contemplates a structure comprising discrete adsorbent
bodies bonded to a **monolithic** substrate through a medium comprising a siloxane
polymer.

DEPR:
Where the invention is embodied in a chromatographic apparatus or solid phase
adsorption device, discrete adsorbent bodies are typically bonded to a
monolithic or essentially **monolithic** substrate, such as an interior wall of a
chromatographic column, or a fiber or contiguous network of fibers that support
the adsorbent particles or a solid phase adsorption device. As used herein,
the term "**monolithic**" includes essentially **monolithic** structures such as a
weave or mat of contiguous fibers.

DEPR:
In certain applications known to the art, a particulate substrate may provide
desirable functions or advantages. It will be understood that, in certain
embodiments, the novel chromatographic apparatus or solid phase adsorption
device of the invention may comprise a substrate which itself comprises
discrete bodies, to which discrete adsorbent bodies are bonded with a siloxane
polymer. There may also be applications other than in chromatography, sample
preparation, or catalysis wherein discrete bodies having a functional surface
property are bonded to a **monolithic** or particulate substrate via the medium of
a siloxane polymer.

CCOR:

DOCUMENT-IDENTIFIER: US 5607580 A
TITLE: Nucleophilic bodies bonded to siloxane and use thereof for separations
from sample matrices

BSPR:
The invention further contemplates a structure comprising discrete adsorbent
bodies bonded to a **monolithic** substrate through a medium comprising a siloxane
polymer.

DEPR:
Where the invention is embodied in a chromatographic apparatus or solid phase
adsorption device, discrete adsorbent bodies are typically bonded to a
monolithic or essentially **monolithic** substrate, such as an interior wall of a
chromatographic column, or a fiber or contiguous network of fibers that support
the adsorbent particles of a solid phase adsorption device. As used herein,
the term "**monolithic**" includes essentially **monolithic** structures such as a
weave or mat of contiguous fibers.

DEPR:
In certain applications known to the art, a particulate substrate may provide
desirable functions or advantages. It will be understood that, in certain
embodiments, the novel chromatographic apparatus or solid phase adsorption
device of the invention may comprise a substrate which itself comprises
discrete bodies, to which discrete adsorbent bodies are bonded with a siloxane
polymer. There may also be applications other than in chromatography, sample
preparation, or catalysis wherein discrete bodies having a functional surface
property are bonded to a **monolithic** or particulate substrate via the medium of
a siloxane polymer.

CCOR:

DOCUMENT-IDENTIFIER: US 5599445 A
TITLE: Nucleophilic bodies bonded to siloxane and use thereof for separations
from sample matrices

BSPR:
The invention further contemplates a structure comprising discrete adsorbent
bodies bonded to a **monolithic** substrate through a medium comprising a siloxane
polymer.

DEPR:
Where the invention is embodied in a chromatographic apparatus or solid phase
adsorption device, discrete adsorbent bodies are typically bonded to a
monolithic or essentially **monolithic** substrate, such as an interior wall of a
chromatographic column, or a fiber or contiguous network of fibers that support
the adsorbent particles of a solid phase adsorption device. As used herein,
the term "**monolithic**" includes essentially **monolithic** structures such as a
weave or mat of contiguous fibers.

DEPR:
In certain applications known to the art, a particulate substrate may provide
desirable functions or advantages. It will be understood that, in certain
embodiments, the novel chromatographic apparatus or solid phase adsorption
device of the invention may comprise a substrate which itself comprises
discrete bodies, to which discrete adsorbent bodies are bonded with a siloxane
linker. There may also be applications other than in chromatography, sample
preparation, or catalysis wherein discrete bodies having a functional surface
property are bonded to a **monolithic** or particulate substrate via the medium of
a siloxane polymer.

CLPR:

1. A structure comprising discrete adsorbent bodies bonded to a monolithic substrate through a medium comprising a polysiloxane polymer.

CCOR:
210/198.2

DOCUMENT-IDENTIFIER: US 5308495 A
TITLE: Chromatography processes using doped sol gel glasses as chromatographic
media

BSPR:
B. Chromatographic Applications: The selective interaction of doped sol-gel
glass with the surrounding chemical compounds makes this type of glasses a
promising chromatographic media. This possibility was specifically mentioned
in the above mentioned patent application. In examples B-F we demonstrate
planar and column chromatographies for liquid and gas applications. **Monolithic**
doped sol-gel glasses can also be used for the same purpose.

DRPR:
FIG. 5 discloses **monolithic** glass detectors doped with ionic surface active
agents to prevent cracking. Upper section: detectors before and after
immersion in solution containing the analyzates; lower row: **monolithic** glass
disks.

DEPR:
FIG. 5 depicts several typical photometric detectors containing doped surface
active agents. The first upper section contains four examples of **monolithic**
doped glasses before and after immersion in aqueous solution containing the
analyzate (from left to right: Redox detector containing Diphenylaminesulfonate
[illegible] and 5 mg cetylpyridinium bromide (CPB) before and after
[illegible] in sodium hypochlorite solution; pH indicator containing
[illegible] and 6.6 mg cetyltrimethylammonium bromide (CTAB)
before and after immersion in basic aqueous solution; iron Detector containing
1.2 mg o-phenanthroline and 5.0 mg CPB before and after immersion in a solution
containing ferrous ammonium sulfate; nitrite indicator containing

4 mg
1-Naphthylenediamine dihydrochloride, 10 mg sulphanilic acid and
6 mg CTAB).
The lower row depicts few detectors (from left to right; copper
detector
containing 1 mg dithiooxamide and 6.25 mg CPB; lead detector
containing 1 mg
galocyanine and 7.5 mg CPB; pH detector containing 5 mg
bromophenol and 6.6 mg
CTAB; nickel detector containing 2 mg dimethyglyoxime and 6.25 mg
CPB; aluminum
detector containing 5 mg alizarin and 12.5 mg CPB)

DEPR:
In all cases the described procedure prevented cracking of the
monolithic
glasses even after few cycles of wetting and drying of the
glasses.

CCXR:
210/198.2

DOCUMENT-IDENTIFIER: US 5141609 A
TITLE: Method and device employing time-delayed integration for detecting
sample components after separation

DEPR:
Charge-Coupled Device. A solid state device comprising an array of detectors
which can be readily adapted for use in the present invention is the
charge-coupled device (CCD). A CCD is a **monolithic** large-format silicon array
detector. Characteristics that make it ideally suited for detection are
extremely high quantum efficiency (.ltoreq.80%), virtually no dark current, and
up to 10.sup.6 individual detector elements in the array. The CCD is
conceptually similar to an electronic photographic film in that both integrate
signal information. The integrating ability of the CCD and lack of dark
current allow the CCD to perform exceptionally well in situations where several
seconds are allowed for integration of the signal. In microcolumn separations,
detection zones can be constructed such that analyte bands are viewed for many
seconds--an ideal measurement task for a CCD.

CCXR:
210/198.2

DOCUMENT-IDENTIFIER: US 4954149 A
TITLE: Injection septum

DEPR:
In addition to the embodiments described above, the present invention provides
for a **monolithic** septum with annular and duckbill ends of a unitary aperture.
Such a septum differs from conventional and other known duckbill seals in that,
when a needle is extended through the septum, the annular aperture and the
duckbill aperture expand elastically to accommodate the needle and press
against the needle under the pressure of their deformation. A wide variety of
elastically deformable materials can be employed in the septum provided by the
present invention. In addition, interlocking of components of a compound
septum can be effected using a variety of means. Dimensions can be changed to
accommodate devices and applications other than those illustrated.

CCXR:
210/198.2

DOCUMENT-IDENTIFIER: US 4792395 A
TITLE: High speed countercurrent centrifuge for removal attachment of
chromatographic columns thereto, and chromatographic column for the same

DEPR:
FIG. 4 shows a variation of the column holder arrangement used in the present
invention. Elements 5' 10' 11', 12', 13', 14', 14a', 16', 18', 19', 20', 21',
22' and 54' correspond, respectively, to the corresponding elements 10, 11, 12,
13, 14, 14a, 16, 18-22 and 54 in the embodiment of FIGS. 1-3 and will not be
further described in detail. In this embodiment, a sleeve 62 is fitted around
central shaft 5'. Thus, flow tubes 54' can extend upwardly through a central
bore in shaft 5', and out through exits 60 and 61, finally connecting to top
flange 20' and tubing adaptors 10'. Quick release nuts 12' and 13' are screw
threaded about sleeve 62. Sleeve 62 is fixed to or is **monolithic** with column
holder 10'. The embodiment of FIG. 4, which places the tubing connections 16'
at the top of spool 19', permits facile inspection of the flow tubes 54' and
their connections. Sleeve 62 is necessary to create space 62a so that the
column 18' may be slid off shaft 5' without hindrance by the portion of tubes
54' extending through opening 60.

CODR:
210/198.2

DOCUMENT-IDENTIFIER: US 4636315 A
TITLE: Fluid separator apparatus and method

DEPR:
While the distributor body has been illustrated as comprising a pair of plates, with the channels conveniently formed at the plates' mating surfaces, it will be appreciated that the distributor body may be formed in other fashions. For example, the distributor body may be formed as a **monolithic** structure with the fluid passages formed therein to communicate between the fluid port and the uniformly spaced distribution openings. The fluid passages on such a **monolithic** structure may be formed by casting or etching techniques known in the art.

CCXR:
210/198.2

DOCUMENT-IDENTIFIER: US 4551251 A
TITLE: **Monolithic** integrated flow circuit

TTL:
Monolithic integrated flow circuit

ABPL:
A **monolithic** multi-channel integrated flow circuit comprising a support matrix sheet or plate impressed or embossed with the desired circuitry; the desired circuit elements such as transfer conduit and separation columns are integral with and defined by the support matrix, which conveniently comprises a first deformable support sheet embossed with the circuits elements by thermoforming techniques, and bonded to a support blank or correspondingly embossed second support sheet to complete and define the circuit.

BSPR:
The invention relates to **monolithic** integrated flow circuits useful in a variety of analytical and preparative applications, and particularly relates to chromatographic flow circuits adaptable to a broad spectrum of known chromatographic separation and concentration processes, including liquid partition chromatography, liquid-solid chromatography, ion-exchange chromatography, gas-liquid chromatography, and gas-solid chromatography.

BSPR:
The invention comprises a **monolithic** multi-channel integrated flow circuit [illegible] fluids. For partitioning fluids according to [illegible] chromatographic principles, the flow circuit of the invention includes a plurality of separation columns or channels interconnected in series by integrated narrower-bore transfer conduits for delivering fluid from one column to a next successive column. The MIFC of the invention

comprises a
support matrix or sheet impressed with the desired circuitry by appropriate
molding or machining techniques; the circuitry is thus integral with and at
least partially defined by the support matrix. Conveniently, the circuit is
fabricated by embossing the circuit in a thin-gauge metal or plastic support
sheet, followed by bonding of the embossed sheet to a support sheet blank to
define the circuit, according to well-known thermoforming techniques, such as
those used for blister packaging. Very complicated integrated flow circuits
can thus readily be produced according to desired pattern in a compact sheet
without prior art adaptors, transfer tubing, joints, and other discrete
elements requiring assembly and structural integrity. Either flexible matrix
sheets, or thicker, more rigid matrix sheets (herein referred to as matrix
"plates") may be employed.

DEPR:
According to the present invention, many complex flow patterns are obtainable
from plastic support sheets or similar matrix material molded to define the
monolithic integrated flow circuits (MIFC) herein described. Characteristic
features of MIFC are as follows:

CLPR:
1. A **monolithic** integrated flow circuit consisting essentially of a deformable
matrix plate having a flow circuit formed therein, said deformable matrix plate
being of formed plastic or metallic thin-gauge sheets, at least one of said
sheets having a pattern formed by embossing thereon, said sheets being bonded
together to form said flow pattern, said pattern defining a plurality of
locules, each of said locules being joined to an adjacent locule by an integral
transfer conduit.

CLPR:

6. A plurality of **monolithic** flow circuits according to claim 1, wherein the
flow circuits formed in said plates are interconnected in series.

CLPR:
8. A plurality of **monolithic** integrated flow circuits according to claim 1,
wherein a plurality of said plates are formed into a block of circuits
interconnected in series.

CLPR:
12. The **monolithic** integrated flow circuit of claim 1, wherein said support
sheets are thin-gauge plastic.

CLPR:
13. The **monolithic** integrated flow circuit of claim 1, wherein said support
sheets are thin-gauge metal.

CLPR:
14. The **monolithic** integrated flow circuit of claim 1, wherein the flow
circuit is embossed in a thermoplastic matrix plate by thermoforming.

CLPP:
16. A method for counter-current chromatography comprising separating a first
liquid from a second liquid with which said first liquid is mixed by passing a
mixture of said first and second liquid through a **monolithic** integrated flow
circuit consisting essentially of a deformable matrix plate having a flow
circuit formed therein, said deformable matrix plate being of formed plastic or
metallic thin-gauge sheets, at least one of said sheets having a pattern formed
by embossing thereon, said sheets being bonded together to form [illegible]
pattern, said pattern defining a plurality of [illegible] of said [illegible]
being joined to an adjacent locule by an integral transfer conduit and
subjecting said mixture to counter-current chromatography in said column.

CCXR:

DOCUMENT-IDENTIFIER: US 4440638 A
TITLE: Surface field-effect device for manipulation of charged species

DEPR:
Dielectric film 96 is suitably a layer of glass. The glass layer serves to hold the top portion of the capacitance device in juxtaposition with the lower portion 100. The glass layer can be formed using conventional sintered infused glass techniques employed in hybrid circuit processing. The capacitive device and measurement system may suitably be in accordance with that disclosed in Sander et al., "MONOLITHIC CAPACITANCE PRESSURE TRANSDUCER-IC WITH PULSE PERIOD OUTPUT", IEEE/Engineering in Medicine and Biology Conference on Frontiers of Engineering and Health Care, 1979, pages 189-192.

CCXR:
210/198.2

DOCUMENT-IDENTIFIER: US 4175037 A
TITLE: Process for packing chromatographic columns

DEPR:
FIG. 2 is a schematic diagram illustrating an apparatus for effecting the method of the present invention used in conjunction with a **monolithic** glass chromatographic column.

DEPR:
Referring to FIG. 2, another embodiment of the present invention is shown wherein a **monolithic** glass chromatographic column 32 is shown being packed in accordance with the method of the present invention. The **monolithic** glass chromatographic column has a continuous passageway formed therethrough which can be packed with chromatographic packing material in accordance with the present invention. Quick-connect and disconnect couplings 34 and 36 enable the column to be connected and disconnected from the packing system of the present invention. Moreover, these couplings enable the packed chromatographic column to be readily connected to gas feed and analytical devices.

CCXR:
210/198.2

DOCUMENT-IDENTIFIER: US 4116636 A
TITLE: Chromatographic column

ABPL:
A **monolithic** glass construction having a continuous passageway formed
therethrough packed with chromatographic packing material is used as a
chromatographic column. The **monolithic** construction enables the column to be
formed of the longest practical length and smallest practical diameter to
increase the efficiency of the column, while also providing a durable column.
Quick-connect and disconnect couplings to a gas feed and analysis device are
mounted on the **monolithic** construction.

BSPR:
In accordance with one form of the invention, the chromatographic column
consists of a **monolithic** glass cylinder having a pair of opposed surfaces and a
side wall in which a continuous helical passageway is formed. The passageway
is packed with gas absorptive material for performing the chromatographic
process. Gas can be injected directly into the helical passageway and a pair
of metal flanges can be inserted over the opposed surface comprising the top
and bottom edges of the glass cylinder.

BSPR:
The flanges can be formed from metal to preclude chipping and breaking of the
monolithic glass construction and to further serve as a conductor to discharge
static electricity which may adhere to the interior passageway in the
monolithic structure. The couplings on the flange also provide a firm means of
support for making the connections to the valve structure.

BSPR:
The **monolithic** glass construction may also take the form of a number of stacked

plates fused together which have continuous interconnected passages of
sinusoidal or other configuration formed therethrough. The passages are also
packed with gas absorptive or adsorptive material.

BSPR:
By using such **monolithic** plates or cylinders, the height of the resulting
column can be constructed at will with any diameter passage within practical
limits, serving to effect a chromatographic column of high efficiency.
Further, the resultant column because of its **monolithic** (or solid one piece)
construction is extremely durable.

BSPR:
The cylindrical column can be formed by wrapping an expendable, sacrificial
tubing in a coil or helix of appropriate spacing and length around a section of
glass tubing supported on a mandrel. It is considered preferable to first cut,
grind, etch or mold the glass tubing in the desired helical configuration or
other desired configuration and to wrap the sacrificial tubing in the resulting
grooved path to thereby establish and retain the desired spacing between the
coils of tubing during subsequent processing. A second tube is then placed
over the coil so a concentric sandwich is formed on the mandrel. Sufficient
heat is applied to one end of the inner and outer tubes to effect fusing of the
tubes at said one end thereof. A vacuum is drawn between the inner and outer
tubes and heat is applied to the tubes, causing said tubes to flow into the
interspacial areas between the sacrificial tubing, thereby fusing the glass
tubes together and forming a **monolithic** structure. When this operation is
complete, the sacrificial tubing is removed by means of an etchant or other
reactant chemical compound such as FeCl.sub.3, HCl, an acetic acid-nitric acid
mixture and the like which creates a continuous passageway of the desired

configuration through the **monolithic** structure. The resultant column is
monolithic (or solid one piece) in structure having the integrity of a single
heavy wall glass tube. The continuous passageway is then loaded with
chromatographic packing thereby forming a **monolithic** packed chromatographic
column.

BSPR:
In the stacked plate construction, sacrificial tubing may be placed in grooves
cut, ground, etched or molded in a desired continuous passageway configuration
on one of the two opposing surfaces of a glass plate and a second plate fused
thereto. After removal of the sacrificial tubing, a **monolithic** plate having a
continuous passageway formed therein is obtained. A plurality of such
monolithic plates may be stacked one on top of the other with the outlet of one
joined to the inlet of the next adjacent plate such as with a glass or metal U
tube. The resulting continuous passageway through the stack of **monolithic**
plates can be packed with chromatographic packing material to create a packed
chromatographic column of significant strength and resistance to breakage.
Moreover, a column obtained in this fashion is surprisingly compact and
efficient.

DETD:
Column 10 includes a **monolithic** glass cylindrical tube 12 having a helical
passageway 14 formed in a side wall 16 thereof. One end of passageway 14 opens
at 15 in the bottom edge 18 of cylinder 12 and the other end of passageway 14
has an opening 20 in the top edge 22 of cylinder 12.

DETD:
Because of the nature of the **monolithic** construction of column 10, durable
columns as long as 200 feet or more may be manufactured having a small diameter
passageway 14 whereby the efficiency of the column may be

substantially
increased. For example, columns having more than 2,000
theoretical plates per
foot have been fabricated with the construction illustrated in
FIGS. 1 to 3
wherein heretofore it was only possible to fabricate columns of
about 800
theoretical plates per foot.

DEFF:
Column 10 is fabricated in a **monolithic** construction by wrapping
an expendable,
sacrificial tubing in a helix of a length and width spacing
between the coils
of the helix as desired around an inner glass tubing section
supported on a
mandrel. It is considered preferable to cut, grind, etch or mold
a grooved
path on the outer surface of the inner glass tubing to serve as a
guide and
retainer for the sacrificial tubing. In this manner, the spacing
between the
wire can be preset and retained during the heating operation. A
second outer
section of glass tubing is placed over the coil and first tube to
form a
concentric sandwich on the supporting mandrel. Sufficient heat
is applied to
one end of the sandwich to effect fusing of the glass tubes at
said one end
thereof. A vacuum is then drawn between the inner and outer
glass tubing to
fuse and flow into the interspacial areas between the sacrificial
tubing. When
this operation is complete, the sacrificial tubing is removed by
means of an
acid etchant or other reactant compound which leaves the desired
configuration
for the column. A chromatographically chemically inert porous
retainer is
inserted into a terminal portion of the [illegible] passageway
[illegible]
column [illegible]. Such retainers
[illegible]
of fiber glass, glass wool, glass frits, [illegible] such
wool [illegible] as
gold wool and the like. Such retainers can also be interposed in
the terminal
portion of the inlet and outlet lines leading to and from the
column to further

protect against loss of packing material. The passageway thus formed by
removal of the sacrificial tubing is packed with an appropriate adsorption-desorption material and a porous retainer as described hereinabove
can be inserted into the initial portion of the continuous passageway of the
column thereby sealing the packing within the passageway of the column. The
resultant structure is **monolithic** in nature having the integrity of a single
heavy wall glass tube of desired length. Moreover, the precision bore of the
passageway obtained through use of the sacrificial tubing is believed to help
in obtaining packing uniformity.

DEPR:
As shown in FIGS. 7 to 9, inclusive, an annular metal flange 24 may be seated
on the bottom and top edges 18 and 22 of cylindrical column 12, respectively.
The metal flanges 24 preclude chipping of the **monolithic** glass construction of
column 12 and can also serve to aid in the elimination of static charges carred
by the sample under analysis in passageway 14.

DEPR:
As shown in FIGS. 4 to 6, inclusive, the chromatographic column 10 can be
formed with both the inlet and outlet for the gas sample in either the top or
bottom edges 18 or 22 of the glass **monolithic** cylinder 12. As shown, the inlet
15 and outlet 20 can be disposed on opposite diametrical portions of the top
edge 22 of cylinder 12. A flange 24 can be disposed over the top edge as well
as the bottom edge, with a quick-disconnect and connect cylindrical coupling 26
[illegible] the inlet 15 and outlet 20. Accordingly, this **monolithic**
construction of chromatographic tube 10 permits convenience for attachment for
suitable analysis equipment.

CLPR:
1. A packed chromatographic column comprising a glass **monolithic** construction

having at least one pair of opposed surfaces and solid glass therebetween;

CLPF:
3. The packed chromatographic column of claim 2 wherein said glass **monolithic** construction is the side wall of a cylindrical tube, said side wall containing said continuous passageway.

CLPF:
11. The packed chromatographic column of claim 1 wherein said glass **monolithic** construction includes at least one substantially planar plate.

CLPF:
14. The packed chromatographic column of claim 13 wherein said glass **monolithic** construction is the side wall of a cylindrical tube, said side wall containing said continuous passageway.

CLPV:
A continuous passageway of essentially constant diameter adapted to receive a fluid therethrough contained wholly within said solid glass between said opposed surfaces of said **monolithic** contruction, the passageway having an inlet and an outlet opening in at least one of said opposed surfaces, and

CCOR:
210/198.2

DOCUMENT-IDENTIFIER: US 5833861 A
TITLE: Perfusive chromatography

DEPR:
Broadly, in accordance with the invention, perfusion chromatography is practiced by passing fluids at velocities above a threshold level through a specially designed matrix characterized by a geometry which is bimodal or **multimodal** with respect to its porosity. Perhaps the most fundamental observation relevant to the new procedure is that it is possible to avoid both the loss of capacity characteristic of convection bound systems and the high plate height and bandspreading characteristics of diffusion bound systems. This can be accomplished by forcing chromatography fluids through a matrix having a set of larger pores, such as are defined by the interstices among a bed of particles, and which determine pressure drops and fluid flow velocities through the bed, and a set of pores of smaller diameter, e.g., anisotropic throughpores. The smaller pores permeate the individual particles and serve to deliver chromatography fluids by convection to surface regions within the particle interactive with the solutes in the chromatography fluid.

DEPR:
From the foregoing description many of the basic engineering goals to be pursued in the fabrication of matrix materials suitable for the practice of perfusion chromatography will be apparent to those skilled in the art. Thus, what is needed to practice perfusion chromatography is a matrix which will not crush under pressure having a bimodal or preferably **multimodal** pore structure and as large a surface area per unit volume as possible. The first and second pore sets which give the material its bimodal flow properties

must have mean
diameters relative to each other so as to permit convective flow through both
sets of pores at high V.sub.beds. The provision of subpores in the matrix is
not required to conduct perfusion chromatography but is preferred because of
the inherent increase in surface area per unit volume of matrix material such a
construction provides.

DEPR:
In contrast to the PL 4,000 material, which, with respect to its pore
structure, is **multimodal,** a more ideal perfusive particle might comprise a
plurality of sets of throughpores and subpores of differing mean diameters. A
bimodal pore size distribution can be achieved in such particle by mixing equal
ratios of particles having two discrete pore sizes or by engineering this
feature at the polymerization stage. Ideally, mean diameter ratio between
throughpore subsets would be less than 10, the mean diameter ratio between the
smallest throughpore sets and the subpores would be less than 20, and the mean
diameter ratio between the first pore set, i.e., the interstices among the
particles making up the matrix, and the largest throughpore subset would be
less than 70, and preferably less than 50. A **multimodal** material might be
produced by agglomerating 500 .ANG. porons to form approximately 1 .mu.m
clusters, which in turn are agglomerated to form 10 .mu.m aggregates, which in
turn may be aggregated to form 100 .mu.m particles. In such a design, the 1
.mu.m clusters would have interstices of a mean diameter in the vicinity of a
few hundred .ANG.. These [illegible] would provide a very high surface area. Diffusive transport within these pores would rarely have to exceed a distance of 0.5 .mu.m or 5,000 .ANG.. Interstices among the 1 .mu.m
clusters making up the 10 .mu.m aggregates would permit convective flow to feed

the diffusive pores. These would be on the order of 0.3 .mu.m in diameter.
These throughpores, in turn would be fed by larger pores defined by
intersticies among the 10 .mu.m particles making up the 100 .mu.m particles.
These would have a mean diameter on the order of 35 .mu.m.

CCXR:
210/198.2

DOCUMENT-IDENTIFIER: US 5605623 A
TITLE: Perfusive chromatography

DEPR:
Broadly, in accordance with the invention, perfusion chromatography is practiced by passing fluids at velocities above a threshold level through a specially designed matrix characterized by a geometry which is bimodal or **multimodal** with respect to its porosity. Perhaps the most fundamental observation relevant to the new procedure is that it is possible to avoid both the loss of capacity characteristic of convection bound systems and the high plate height and bandspreading characteristics of diffusion bound systems. This can be accomplished by forcing chromatography fluids through a matrix having a set of larger pores, such as are defined by the interstices among a bed of particles, and which determine pressure drops and fluid flow velocities through the bed, and a set of pores of smaller diameter, e.g., anisotropic throughpores. The smaller pores permeate the individual particles and serve to deliver chromatography fluids by convection to surface regions within the particle interactive with the solutes in the chromatography fluid.

DEPR:
From the foregoing description many of the basic engineering goals to be pursued in the fabrication of matrix materials suitable for the practice of perfusion chromatography will be apparent to those skilled in the art. Thus, what is needed to practice perfusion chromatography is a matrix which will not crush under pressure having a bimodal or preferably **multimodal** pore structure and as large a surface area per unit volume as possible. The first and second pore sets which give the material its bimodal flow properties

must have mean
diameters relative to each other so as to permit convective flow through both
sets of pores at high V.sub.beds. The provision of subpores in the matrix is
not required to conduct perfusion chromatography but is preferred because of
the inherent increase in surface area per unit volume of matrix material such a
construction provides.

DEPR:
In contrast to the PL 4,000 material, which, with respect to its pore
structure, is **multimodal,** a more ideal perfusive particle might comprise a
plurality of sets of throughpores and subpores of differing mean diameters. A
bimodal pore size distribution can be achieved in such particle by mixing equal
ratios of particles having two discrete pore sizes or by engineering this
feature at the polymerization stage. Ideally, mean diameter ratio between
throughpore subsets would be less than 10, the mean diameter ratio between the
smallest throughpore sets and the subpores would be less than 20, and the mean
diameter ratio between the first pore set, i.e., the intersticies among the
particles making UP the matrix, and the largest throughpore subset would be
less than 70, and preferably less than 50. A **multimodal** material might be
produced by agglomerating 500 .ANG. porons to form approximately 1 .mu.m
clusters, which in turn are agglomerated to form 10 .mu.m aggregates, which in
turn may be aggregated to form 100 .mu.m particles. In such a [illegible] the 1
.mu.m clusters would have interstices of a mean diameter in the vicinity of a
few hundred .ANG.. These [illegible] provide a relatively high
surface area. Diffusive transport within these pores would rarely have to
exceed a distance of 0.5 .mu.m or 5,000 .ANG.. Intersticies among the 1 .mu.m
clusters making up the 10 .mu.m aggregates would permit convective flow to feed

the diffusive pores. These would be on the order of 0.3 .mu.m in diameter.
These throughpores, in turn would be fed by larger pores defined by
intersticies among the 10 .mu.m particles making up the 100 .mu.m particles.
These would have a mean diameter on the order of 35 .mu.m.

CLPR:
8. The chromatography system of claim 7 wherein the packed particles define a
bimodal or **multimodal** pore structure.

CCOR:
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DOCUMENT-IDENTIFIER: US 5552041 A
TITLE: Perfusive chromatography

DEPR:
Broadly, in accordance with the invention, perfusion chromatography is practiced by passing fluids at velocities above a threshold level through a specially designed matrix characterized by a geometry which is bimodal or **multimodal** with respect to its porosity. Perhaps the most fundamental observation relevant to the new procedure is that it is possible to avoid both the loss of capacity characteristic of convection bound systems and the high plate height and bandspreading characteristics of diffusion bound systems. This can be accomplished by forcing chromatography fluids through a matrix having a set of larger pores, such as are defined by the interstices among a bed of particles, and which determine pressure drops and fluid flow velocities through the bed, and a set of pores of smaller diameter, e.g., anisotropic throughpores. The smaller pores permeate the individual particles and serve to deliver chromatography fluids by convection to surface regions within the particle interactive with the solutes in the chromatography fluid.

DEPR:
From the foregoing description many of the basic engineering goals to be pursued in the fabrication of matrix materials suitable for the practice of perfusion chromatography will be apparent to those skilled in the art. Thus, what is needed to practice perfusion chromatography is a matrix which will not crush under pressure having a bimodal or preferably **multimodal** pore structure and as large a surface area per unit volume as possible. The first and second pore sets which give the material its bimodal flow properties

must have mean
diameters relative to each other so as to permit convective flow through both
sets of pores at high V.sub.beds. The provision of subpores in the matrix is
not required to conduct perfusion chromatography but is preferred because of
the inherent increase in surface area per unit volume of matrix material such a
construction provides.

DEPR:
In contrast to the PL 4,000 material, which, with respect to its pore
structure, is **multimodal,** a more ideal perfusive particle might comprise a
plurality of sets of throughpores and subpores of differing mean diameters. A
bimodal pore size distribution can be achieved in such particle by mixing equal
ratios of particles having two discrete pore sizes or by engineering this
feature at the polymerization stage. Ideally, mean diameter ratio between
throughpore subsets would be less than 10, the mean diameter ratio between the
smallest throughpore sets and the subpores would be less than 20, and the mean
diameter ratio between the first pore set, i.e., the interstices among the
particles making up the matrix, and the largest throughpore subset would be
less than 70, and preferably less than 50. A **multimodal** material might be
produced by agglomerating 500 .ANG. porons to form approximately 1 .mu.m
clusters, which in turn are agglomerated to form 10 .mu.m aggregates, which in
turn may be aggregated to form 100 .mu.m particles. In such a [illegible]
.mu.m clusters would have interstices of a mean diameter in the vicinity of a
few hundred .ANG.. These pores provide the majority of the
surface area. Diffusive transport within these pores would rarely have to
exceed a distance of 0.5 .mu.m or 5,000 .ANG.. Interstices among the 1 .mu.m
clusters making up the 10 .mu.m aggregates would permit convective flow to feed

the diffusive pores. These would be on the order of 0.3 .mu.m in diameter.
These throughpores, in turn would be fed by larger pores defined by
intersticies among the 10 .mu.m particles making up the 100 .mu.m particles.
These would have a mean diameter on the order of 35 .mu.m.

CCOR:
210/198.2

DOCUMENT-IDENTIFIER: US 5540834 A
TITLE: Synthesis of porous inorganic particles by polymerization-induced colloid aggregation (PICA)

DEPR:
The specific surface area and porosity of the sintered particles measured by nitrogen adsorption were 13 m.sup.2 /g and 29%, respectively, which are in reasonable agreement with close-packed, dense ZrO.sub.2 spheres with nonuniform particle size. The pore size distributions (psd) obtained by nitrogen adsorption and desorption, and mercury porosimetry (intrusion) are displayed in FIGS. 4A, 4B and FIG. 5, respectively. From N.sub.2 adsorption, the psd was determined to be rather narrow with a maximum near 400 .ANG. and a small contribution of pores larger than 500 .ANG. and smaller than 100 .ANG.. From N.sub.2 desorption, the psd was determined to be **multimodal** with nearly all pores between 100 .ANG. and 200 .ANG. in diameter and some pores below 50 .ANG.. This discrepancy is mainly due to pore blocking or network effects, whereby desorption from a pore in a network is influenced by the state of the neighboring pores. Nitrogen adsorption probes the main channel size and can be considered free of pore blocking effects, while nitrogen desorption shows a disproportionately large amount of small pores due to "bottle necks." The psd obtained from mercury porosimetry (intrusion) are also influenced by pore-interconnections; as shown in FIG. 5, it is broad with pores between approximately 125 .ANG. and 300 .ANG. in diameter and a maximum near 200 .ANG., in reasonable agreement with the psd from N.sub.2 desorption.

DEPR:
The pore-size distributions (psd's) after sintering are shown in

FIG. 19. All samples exhibited **multimodal** psd's with pore diameters ranging between 100 .ANG. and 450 .ANG.. The sample synthesized at pH 1.2 contained some hollow particles but its psd seemed qualitatively similar to those of samples synthesized at higher pH (non-hollow particles). This is because N.sub.2 adsorption only probes the pores within the ZrO.sub.2 shells and not the large voids they encompass. Surface areas and porosities (.epsilon..sub.particle) for these samples are listed in Table 3. Note that there is qualitative agreement with FIG. 12E, but quantitative agreement is not expected since condensing N.sub.2 cannot be used to distinguish between hollow cores and interstitial volume between aggregates. The N.sub.2 psd's show that the higher the pH, the greater contribution of small pores to the total porosity.

CCOR:
210/198.2

DOCUMENT-IDENTIFIER: US 5522994 A
TITLE: Single column chromatographic determination of small molecules in
mixtures with large molecules

CCXR:
210/198.2

ORPL:
Little, "Sequential **Multimodal** Elution for Pseudomultidimensional Liquid
Chromatography on a Single Column," Anal. Chem., 63 (1991) pp. 33-34.

DOCUMENT-IDENTIFIER: US 5431807 A
TITLE: **Multimodal** chromatographic separation media and process for using same

TTL:
Multimodal chromatographic separation media and process for using same

ABPL:
A process for carrying out in a consecutive fashion different modes of chromatographic separation in a liquid chromatography column using a single separation medium is disclosed. Separation media for use in such **multimodal** separations are also disclosed.

BSPR:
It may be possible to use combinations of different separation media in different columns for **multimodal** separations. An example of this multiple column bimodal separation was described recently by Wheatley J. B., J. Chromatogr., 603 (1992) 273. The bimodal separation of small molecules in one column packed with one separation medium and based on sequential **multimodal** elution was described by Little E. L., Jeansonne M. S., Foley J. P.; Anal Chem., 63, 1991, 33. They combined ion-exchange and reversed phase chromatography for the separation of a complex sample containing two groups of compounds: charged and non-polar. The use of two different gradients, i.e. a pH gradient and a methanol gradient, resulted in the separation of the charged molecules first, followed by the separ

DOCUMENT-IDENTIFIER: US 5833861 A
TITLE: Perfusive chromatography

DEPR:
Broadly, in accordance with the invention, perfusion chromatography is practiced by passing fluids at velocities above a threshold level through a specially designed matrix characterized by a geometry which is bimodal or multimodal with respect to its porosity. Perhaps the most fundamental observation relevant to the new procedure is that it is possible to avoid both the loss of capacity characteristic of convection bound systems and the high plate height and bandspreading characteristics of diffusion bound systems. This can be accomplished by forcing chromatography fluids through a matrix having a set of larger pores, such as are defined by the interstices among a bed of particles, and which determine pressure drops and fluid flow velocities through the bed, and a set of pores of smaller diameter, e.g., anisotropic throughpores. The smaller pores permeate the individual particles and serve to deliver chromatography fluids by convection to surface regions within the particle interactive with the solutes in the chromatography fluid.

DEPR:
From the foregoing description many of the basic engineering goals to be pursued in the fabrication of matrix materials suitable for the practice of perfusion chromatography will be apparent to those skilled in the art. Thus, what is needed to practice perfusion chromatography is a matrix which will not crush under pressure having a bimodal or preferably multimodal pore structure and as large a surface area per unit volume as possible. The first and second pore sets which give the material its bimodal flow properties

must have mean
diameters relative to each other so as to permit convective flow through both
sets of pores at high V.sub.beds. The provision of subpores in the matrix is
not required to conduct perfusion chromatography but is preferred because of
the inherent increase in surface area per unit volume of matrix material such a
construction provides.

DEPR:
In contrast to the PL 4,000 material, which, with respect to its pore
structure, is **multimodal,** a more ideal perfusive particle might comprise a
plurality of sets of throughpores and subpores of differing mean diameters. A
bimodal pore size distribution can be achieved in such particle by mixing equal
ratios of particles having two discrete pore sizes or by engineering this
feature at the polymerization stage. Ideally, mean diameter ratio between
throughpore subsets would be less than 10, the mean diameter ratio between the
smallest throughpore sets and the subpores would be less than 20, and the mean
diameter ratio between the first pore set, i.e., the interstices among the
particles making up the matrix, and the largest throughpore subset would be
less than 70, and preferably less than 50. A **multimodal** material might be
produced by agglomerating 500 .ANG. pores to form approximately 1 .mu.m
clusters, which in turn are agglomerated to form 10 .mu.m aggregates, which in
turn may be aggregated to form 100 .mu.m particles. In such a design, the 1
.mu.m clusters would have interstices [illegible] mean diameter in the vicinity of a
few hundred .ANG. [illegible] subpores and provide a very high
surface area. Diffusive transport within these pores will rarely have to
exceed a distance of 0.5 .mu.m or 5,000 .ANG.. Interstices among the 1 .mu.m
clusters making up the 10 .mu.m aggregates would permit convective flow to feed

the diffusive pores. These would be on the order of 0.3 .mu.m in diameter.
These throughpores, in turn would be fed by larger pores defined by
intersticies among the 10 .mu.m particles making up the 100 .mu.m particles.
These would have a mean diameter on the order of 35 .mu.m.

CCXR:
210/198.2

DOCUMENT-IDENTIFIER: US 5605623 A
TITLE: Perfusive chromatography

DEPR:
Broadly, in accordance with the invention, perfusion chromatography is practiced by passing fluids at velocities above a threshold level through a specially designed matrix characterized by a geometry which is bimodal or **multimodal** with respect to its porosity. Perhaps the most fundamental observation relevant to the new procedure is that it is possible to avoid both the loss of capacity characteristic of convection bound systems and the high plate height and bandspreading characteristics of diffusion bound systems. This can be accomplished by forcing chromatography fluids through a matrix having a set of larger pores, such as are defined by the intersticies among a bed of particles, and which determine pressure drops and fluid flow velocities through the bed, and a set of pores of smaller diameter, e.g., anisotropic throughpores. The smaller pores permeate the individual particles and serve to deliver chromatography fluids by convection to surface regions within the particle interactive with the solutes in the chromatography fluid.

DEPR:
From the foregoing description many of the basic engineering goals to be [illegible] fabrication of matrix materials suitable for the practice of [illegible] will be apparent to those skilled in the art. Thus, what is needed is plastics perfusion chromatography is a matrix which will not crush under pressure having a bimodal or preferably **multimodal** pore structure and as large a surface area per unit volume as possible. The first and second pore sets which give the material its bimodal flow properties

must have mean
diameters relative to each other so as to permit convective flow through both
sets of pores at high V.sub.beds. The provision of subpores in the matrix is
not required to conduct perfusion chromatography but is preferred because of
the inherent increase in surface area per unit volume of matrix material such a
construction provides.

DEPR:
In contrast to the PL 4,000 material, which, with respect to its pore
structure, is **multimodal,** a more ideal perfusive particle might comprise a
plurality of sets of throughpores and subpores of differing mean diameters. A
bimodal pore size distribution can be achieved in such particle by mixing equal
ratios of particles having two discrete pore sizes or by engineering this
feature at the polymerization stage. Ideally, mean diameter ratio between
throughpore subsets would be less than 10, the mean diameter ratio between the
smallest throughpore sets and the subpores would be less than 20, and the mean
diameter ratio between the first pore set, i.e., the intersticies among the
particles making UP the matrix, and the largest throughpore subset would be
less than 70, and preferably less than 50. A **multimodal** material might be
produced by agglomerating 500 .ANG. porons to form approximately 1 .mu.m
clusters, which in turn are agglomerated to form 10 .mu.m aggregates, which in
turn may be aggregated to form 100 .mu.m particles. In such a
[illegible]
.mu.m clusters would have interstices of a mean diameter in the
[illegible]
few hundred .ANG.. These would define [illegible]
very high
surface area. Diffusive transport within these pores would rarely have to
exceed a distance of 0.5 .mu.m or 5,000 .ANG.. Interstices among the 1 .mu.m
clusters making up the 10 .mu.m aggregates would permit convective flow to feed

the diffusive pores. These would be on the order of 0.3 .mu.m in diameter.
These throughpores, in turn would be fed by larger pores defined by
intersticies among the 10 .mu.m particles making up the 100 .mu.m particles.
These would have a mean diameter on the order of 35 .mu.m.

CLPR:
8. The chromatography system of claim 7 wherein the packed particles define a
bimodal or **multimodal** pore structure.

CCOR:
210/198.2

DOCUMENT-IDENTIFIER: US 5552041 A
TITLE: Perfusive chromatography

DEPR:
Broadly, in accordance with the invention, perfusion chromatography is practiced by passing fluids at velocities above a threshold level through a specially designed matrix characterized by a geometry which is bimodal or **multimodal** with respect to its porosity. Perhaps the most fundamental observation relevant to the new procedure is that it is possible to avoid both the loss of capacity characteristic of convection bound systems and the high plate height and bandspreading characteristics of diffusion bound systems. This can be accomplished by forcing chromatography fluids through a matrix having a set of larger pores, such as are defined by the interstices among a bed of particles, and which determine pressure drops and fluid flow velocities through the bed, and a set of pores of smaller diameter, e.g., anisotropic throughpores. The smaller pores permeate the individual particles and serve to deliver chromatography fluids by convection to surface regions within the particle interactive with the solutes in the chromatography fluid.

DEPR:
From the foregoing description many of the basic engineering goals to be [illegible] the fabrication of matrix materials suitable for the practice of [illegible] chromatography will be apparent to those skilled in the art. Thus, what is needed to practice perfusion chromatography is a matrix which will not crush under pressure having a bimodal or preferably **multimodal** pore structure and as large a surface area per unit volume as possible. The first and second pore sets which give the material its bimodal flow properties

must have mean
diameters relative to each other so as to permit convective flow through both
sets of pores at high V.sub.beds. The provision of subpores in the matrix is
not required to conduct perfusion chromatography but is preferred because of
the inherent increase in surface area per unit volume of matrix material such a
construction provides.

DEPR:
In contrast to the PL 4,000 material, which, with respect to its pore
structure, is **multimodal,** a more ideal perfusive particle might comprise a
plurality of sets of throughpores and subpores of differing mean diameters. A
bimodal pore size distribution can be achieved in such particle by mixing equal
ratios of particles having two discrete pore sizes or by engineering this
feature at the polymerization stage. Ideally, mean diameter ratio between
throughpore subsets would be less than 10, the mean diameter ratio between the
smallest throughpore sets and the subpores would be less than 20, and the mean
diameter ratio between the first pore set, i.e., the interstices among the
particles making up the matrix, and the largest throughpore subset would be
less than 70, and preferably less than 50. A **multimodal** material might be
produced by agglomerating 500 .ANG. porons to form approximately 1 .mu.m
clusters, which in turn are agglomerated to form 10 .mu.m aggregates, which in
turn may be aggregated to form 100 .mu.m particles. In such a design, the 1
.mu.m clusters would have interstices of a mean diameter in the vicinity of
few hundred .ANG.. These would define the subpore [illegible] very high
surface area. Diffusive transport within these pores would rarely have to
exceed a distance of 0.5 .mu.m or 5,000 .ANG.. Interstices among the 1 .mu.m
clusters making up the 10 .mu.m aggregates would permit convective flow to feed

the diffusive pores. These would be on the order of 0.3 .mu.m in diameter.
These throughpores, in turn would be fed by larger pores defined by
intersticies among the 10 .mu.m particles making up the 100 .mu.m particles.
These would have a mean diameter on the order of 35 .mu.m.

CCOR:
210/198.2

DOCUMENT-IDENTIFIER: US 5540834 A
TITLE: Synthesis of porous inorganic particles by polymerization-induced colloid aggregation (PICA)

DEPR:
The specific surface area and porosity of the sintered particles measured by nitrogen adsorption were 13 m.sup.2 /g and 29%, respectively, which are in reasonable agreement with close-packed, dense ZrO.sub.2 spheres with nonuniform particle size. The pore size distributions (psd) obtained by nitrogen adsorption and desorption, and mercury porosimetry (intrusion) are displayed in FIGS. 4A, 4B and FIG. 5, respectively. From N.sub.2 adsorption, the psd was determined to be rather narrow with a maximum near 400 .ANG. and a small contribution of pores larger than 500 .ANG. and smaller than 100 .ANG.. From N.sub.2 desorption, the psd was determined to be **multimodal** with nearly all pores between 100 .ANG. and 200 .ANG. in diameter and some pores below 50 .ANG.. This discrepancy is mainly due to pore blocking or network effects, whereby desorption from a pore in a network is influenced by the state of the neighboring pores. Nitrogen adsorption probes the main channel size and can be considered free of pore blocking effects, while nitrogen desorption shows a disproportionately large amount of small pores due to "bottle necks." The psd obtained from mercury porosimetry (intrusion) are also influenced by pore interconnections; as shown in FIG. 5, it is broad with pores between approximately 125 .ANG. and 300 .ANG. in diameter and a maximum near 200 .ANG., in reasonable agreement with the psd from N.sub.2 desorption.

DEPR:
The pore-size distributions (psd's) after sintering are shown in

FIG. 19. All samples exhibited **multimodal** psd's with pore diameters ranging between 100 .ANG. and 450 .ANG.. The sample synthesized at pH 1.2 contained some hollow particles but its psd seemed qualitatively similar to those of samples synthesized at higher pH (non-hollow particles). This is because N.sub.2 adsorption only probes the pores within the ZrO.sub.2 shells and not the large voids they encompass. Surface areas and porosities (.epsilon..sub.particle) for these samples are listed in Table 3. Note that there is qualitative agreement with FIG. 12E, but quantitative agreement is not expected since condensing N.sub.2 cannot be used to distinguish between hollow cores and interstitial volume between aggregates. The N.sub.2 psd's show that the higher the pH, the greater contribution of small pores to the total porosity.

CODE:
210/198.2

DOCUMENT-IDENTIFIER: US 5522994 A
TITLE: Single column chromatographic determination of small molecules in
mixtures with large molecules

CCXR:
210/198.2

ORPL:
Little, "Sequential **Multimodal** Elution for Pseudomultidimensional Liquid
Chromatography on a Single Column," Anal. Chem., 63 (1991) pp. 33-34.

DOCUMENT-IDENTIFIER: US 5431807 A
TITLE: **Multimodal** chromatographic separation media and process for using same

TTL:
Multimodal chromatographic separation media and process for using same

ABPL:
A process for carrying out in a consecutive fashion different modes of
chromatographic separation in a liquid chromatography column using a single
separation medium is disclosed. Separation media for use in such **multimodal**
separations are also disclosed.

BSPS:
It may be possible to use combinations of different separation media in
different columns for **multimodal** separations. An example of this multiple
column bimodal separation was described recently by Wheatley J. B., J.
Chromatogr., 603 (1992) 279. The bimodal separation of small molecules in one
column packed with one separation medium and based on sequential **multimodal**
elution was described by Little E. L., Jeansonne M. S., Foley J. P.; Anal
Chem., 63, 1991, 33. They combined ion-exchange and reversed phase
chromatography for the separation of a complex sample containing two groups of
compounds: charged and non-polar. The use of two different gradients, i.e. a
[illegible] and a methanol gradient, resulted in the separation of the charged
[illegible], followed by the separation of the neutral molecules after
[illegible] the acetonitrile phase. This approach makes use of imperfect
surface functionalization of porous silica beads which contained C.sub.1,
C.sub.8 or C.sub.18 groups together with the original acidic surface silanol
groups. Similarly, the DIONEX OmniPack PAX-500 column is packed

with
non-porous poly[styrene-divinylbenzene] beads coated on the bead surface with
attached ion-exchange latex particles (as described by the DIONEX booklet).
Here again, the coating of the bead surface is imperfect and it is the
non-covered hydrophobic areas of the original non-porous beads that are used
for separation in the second mode. This approach excludes combinations not
involving the reversed phase mode (the original ST-DVB surface remains
non-polar even after attachment of latex particles) as well as any size
exclusion separation.

BSPR:
This **multimodal** separation process is able to achieve separation in a single
column in a consecutive operation because of the properties of the separation
medium. The separation medium generally comprises a porous material which has
been pretreated so that it has at least two different types of surface groups
which have different functionalities. These different surface groups are
disposed in different size range pores within the porous material. Pore size
as used herein can mean a single measured average size, for example, 25 nm, but
in most cases it means a particular range of sizes, for example, 50-500 nm. An
example of such a porous material of the present invention is one wherein there
are hydrophilic surface groups in pores having a size of from about 5-25 nm and
hydrophobic surface groups in pores ranging in a size of from about 25-50 nm.
Another example is a material having hydrophilic groups in pores below 25 nm in
size and hydrophobic groups in pores above 25 nm in size. As
the different functionalities of the surface groups, materials that have
affinities to such different surface groups may be separated during different
modes of separation, which may be carried out in a consecutive fashion. As

used herein, different molecules means molecules of different sizes, different chemical affinities, different structures, compositions, polarities, chiralities, activities, etc.

DRPR:
The key in selecting a mobile phase for a **multimodal** separation is the consecutive use of mobile phases, in each individual mode, that do not interfere with the absorption of compounds to be separated in any of the subsequent modes. Otherwise, the separation will not be **multimodal** and one group of compounds will leave the column without any separation, as documented in Example 6 and FIG. 7. Under this assumption, even a mobile phase for trimodal separation is easily designed by a person skilled in the art of liquid chromatography.

DRPR:
Reaction schemes 1-4 not only describe the particular sets of reactions leading to **multimodal** separation media but they also show the concepts of making such media in general. The starting polymer must be porous with relatively broad pore size distribution and possess reactive groups on the surface of the pores. Typically, the pore-size selectivity of the modification reactions are controlled by the molecular weight of the catalyst or reagent used in the particular modifying reaction and by the solvent. The number of modes accommodated in a separation medium is theoretically not limited but practically will rarely exceed three. The most important part in designing the [illegible] **multimodal** [illegible] right choice of the path. The product of a given reaction affecting pores of a given size should not affect the groups already built up in the previous reaction step within pores of a different size.

DRPR:
The **multimodal** separation process of the present invention may even use very tiny differences between the separation modes as is the case with reversed phase and hydrophobic interaction chromatography. The separation medium can be prepared by a set of reactions shown in Reaction Scheme 5.

CLPR:
1. A **multimodal** separation medium for use in liquid chromatography comprising a porous separation medium having at least two different pore size ranges with each pore size range containing a different surface group, having a different functionality compared to the surface groups in the other pore size range, said porous separation medium being capable of separating molecules in a sample added to a chromatography column containing said separation medium during different modes of separation which are carried out in a consecutive fashion using a single separation medium.

CLPR:
2. A **multimodal** separation medium for use in chromatography comprising a porous separation medium having at least two ranges of pore size, with each range of pore sizes having surface groups of a chemical composition different from that of other pore size ranges.

CCOR:
210/198.2

ORPL:
Hira, "Sequential **Multimodal** Elution for Pseudomultidimensional Liquid [illegible] in a Single Column," Anal. Chem., 63 (1991) pp. 33-44.

DOCUMENT-IDENTIFIER: US 5384042 A
TITLE: Perfusive chromatography

DEPR:
Broadly, in accordance with the invention, perfusion chromatography is practiced by passing fluids at velocities above a threshold level through a specially designed matrix characterized by a geometry which is bimodal or **multimodal** with respect to its porosity. Perhaps the most fundamental observation relevant to the new procedure is that it is possible to avoid both the loss of capacity characteristic of convection bound systems and the high plate height and bandspreading characteristics of diffusion bound systems. This can be accomplished by forcing chromatography fluids through a matrix having a set of larger pores, such as are defined by the interstices among a bed of particles, and which determine pressure drops and fluid flow velocities through the bed, and a set of pores of smaller diameter, e.g., anisotropic throughpores. The smaller pores permeate the individual particles and serve to deliver chromatography fluids by convection to surface regions within the particle interactive with the solutes in the chromatography fluid.

DEPR:
From the foregoing description many of the basic engineering goals to be pursued in the fabrication of matrix materials suitable for the practice of perfusion chromatography will be apparent to those skilled in the art. Thus, what is needed to practice perfusion chromatography is a matrix which will not crush under pressure having a bimodal or preferably **multimodal** pore structure and as large a surface area per unit volume as possible. The first and second pore sets which give the material its bimodal flow properties

must have mean
diameters relative to each other so as to permit convective flow through both
sets of pores at high V.sub.beds. The provision of subpores in the matrix is
not required to conduct perfusion chromatography but is preferred because of
the inherent increase in surface area per unit volume of matrix material such a
construction provides.

DEPR:
In contrast to the PL 4,000 material, which, with respect to its pore
structure, is **multimodal,** a more ideal perfusive particle might comprise a
plurality of sets of throughpores and subpores of differing mean diameters. A
bimodal pore size distribution can be achieved in such particle by mixing equal
ratios of particles having two discrete pore sizes or by engineering this
feature at the polymerization stage. Ideally, mean diameter ratio between
throughpore subsets would be less than 10, the mean diameter ratio between the
smallest throughpore sets and the subpores would be less than 20, and the mean
diameter ratio between the first pore set, i.e., the interstices among the
particles making up the matrix, and the largest throughpore subset would be
less than 70, and preferably less than 50. A **multimodal** material might be
produced by agglomerating 500 .ANG. porons to form approximately 1 .mu.m
clusters, which in turn are agglomerated to form 10 .mu.m aggregates, which in
turn may be aggregated to form 100 .mu.m particles. In such a design, the 1
.mu.m clusters would have interstices [illegible] in the vicinity of
[illegible] a very high
surface area. Diffusive transport within these pores would rarely have to
exceed a distance of 0.5 .mu.m or 5,000 .ANG.. Interstices among the 1 .mu.m
clusters making up the 10 .mu.m aggregates would permit convective flow to feed

the diffusive pores. These would be on the order of 0.3 .mu.m in diameter.
These throughpores, in turn would be fed by larger pores defined by
intersticies among the 10 .mu.m particles making up the 100 .mu.m particles.
These would have a mean diameter on the order of 35 .mu.m.

CLPR:
22. A matrix for conducting high efficiency adsorption chromatography of
biological molecules, the matrix comprising a packed bed of rigid, polystyrene
divinylbenzene particles having a mean diameter between 10 and 20 micrometers,
defining a bimodal or **multimodal** pore structure and comprising chemically
active regions linked to the surface of the particles for reversibly sorbing
biological molecules, said matrix being characterized in that

CLPV:
a packed bed of rigid particles comprising an inorganic material, the particles
having a mean diameter within the range of 20 micrometers to 100 micrometers
and defining a bimodal or **multimodal** pore structure, one set of pores being
particle transecting throughpores having a mean diameter greater than at least
4000 .ANG., another set of pores being subpores in fluid communication with the
throughpores, and, disposed within at least said subpores, reactive groups
comprising one of anionic sulfonate groups, cationic quaternary ammonium
groups, immunoglobulines, or hydrocarbons, the ratio of the means diameter of
the particles to the mean diameter of a throughpores passing through the
particle being sufficient to permit convective transport of
[illegible]
chromatographic
separation so that, at flow rates greater than [illegible] cm./hr., the rate of
biological molecule transport into the throughpores is dependent on the
velocity of liquid passing through the bed, and sorption capacity remains

substantially constant over a range of flowrates.

CCOR:
210/198.2

DOCUMENT-IDENTIFIER: US 5316680 A
TITLE: **Multimodal** chromatographic separation media and process for using same

TTL:
Multimodal chromatographic separation media and process for using same

ABPL:
A process for carrying out in a consecutive fashion different modes of
chromatographic separation in a liquid chromatography column using a single
separation medium is disclosed. Separation media for use in such **multimodal**
separations are also disclosed.

BSPR:
It may be possible to use combinations of different separation media in
different columns for **multimodal** separations. An example of this multiple
column bimodal separation was described recently by Wheatley J. B., J.
Chromatogr., 603 (1992) 273. The bimodal separation of small molecules in one
column packed with one separation medium and based on sequential **multimodal**
elution was described by Little E. L., Jeansonne M. S., Foley J. P.; Anal
Chem., 63, 1991, 33. They combined ion-exchange and reversed phase
chromatography for the separation of a complex sample containing two groups of
compounds: charged and non-polar. The use of two different gradients, i.e. a
pH gradient and a methanol gradient, resulted in the separation of the charged
[illegible] and by the separation of the neutral molecules after
switching to the second mobile phase. This approach makes use of imperfect
surface functionalization of porous silica beads which contained C.sub.1,
C.sub.8 or C.sub.18 groups together with the original acidic surface silanol
groups. Similarly, the DIONEX OmniPack PAX-500 column is packed

with
non-porous poly[styrene-divinylbenzene] beads coated on the bead surface with
attached ion-exchange latex particles (as described by the DIONEX booklet).
Here again, the coating of the bead surface is imperfect and it is the
non-covered hydrophobic areas of the original non-porous beads that are used
for separation in the second mode. This approach excludes combinations not
involving the reversed phase mode (the original ST-DVB surface remains
non-polar even after attachment of latex particles) as well as any size
exclusion separation.

BSPR:
This **multimodal** separation process is able to achieve separation in a single
column in a consecutive operation because of the properties of the separation
medium. The separation medium generally comprises a porous material which has
been pretreated so that it has at least two different types of surface groups
which have different functionalities. These different surface groups are
disposed in different size range pores within the porous material. Pore size
as used herein can mean a single measured average size, for example, 25 nm, but
in most cases it means a particular range of sizes, for example, 50-500 nm. An
example of such a porous material of the present invention is one wherein there
are hydrophilic surface groups in pores having a size of from about 5-25 nm and
hydrophobic surface groups in pores ranging in a size of from about [illegible].
Another example is a material having hydrophilic groups in pores [illegible]
size and hydrophobic groups in pores above [illegible] to result in
the different functionalities of the surface groups, molecules that have
affinities to such different surface groups may be separated during different
modes of separation, which may be carried out in a consecutive fashion. As

used herein, different molecules means molecules of different sizes, different chemical affinities, different structures, compositions, polarities, chiralities, activities, etc.

DEPR:
The key in selecting a mobile phase for a **multimodal** separation is the consecutive use of mobile phases, in each individual mode, that do not interfere with the absorption of compounds to be separated in any of the subsequent modes. Otherwise, the separation will not be **multimodal** and one group of compounds will leave the column without any separation, as documented in Example 6 and FIG. 7. Under this assumption, even a mobile phase for trimodal separation is easily designed by a person skilled in the art of liquid chromatography.

DEPR:
Reaction Schemes 1-4 not only describe the particular sets of reactions leading to **multimodal** separation media but they also show the concepts of making such media in general. The starting polymer must be porous with relatively broad pore size distribution and possess reactive groups on the surface of the pores. Typically, the pore-size selectivity of the modification reactions are controlled by the molecular weight of the catalyst or reagent used in the particular modifying reaction and by the solvent. The number of modes accommodated in a separation medium is theoretically not limited but practically will rarely exceed three. The most important part in designing the reaction strategy for preparation of a **multimodal** medium is the first step of the path. The product of a given reaction affecting pores of a given size should not affect the groups already built up in the previous reaction step within pores of a different size.

DEPR:
The **multimodal** separation process of the present invention may even use very
tiny differences between the separation modes as is the case with reversed
phase and hydrophobic interaction chromatography. The separation medium can be
prepared by a set of reactions shown in Reaction Scheme 5.

CCXR:
210/198.2

ORPL:
Little, "Sequential **Multimodal** Elution for Pseudomultidimensional Liquid
Chromatography on a Single Column," Anal. Chem., 63, (1991) pp. 33-44.

DOCUMENT-IDENTIFIER: US 5228989 A
TITLE: Perfusive chromatography

DEPR:
Broadly, in accordance with the invention, perfusion chromatography is practiced by passing fluids at velocities above a threshold level through a specially designed matrix characterized by a geometry which is bimodal or **multimodal** with respect to its porosity. Perhaps the most fundamental observation relevant to the new procedure is that it is possible to avoid both the loss of capacity characteristic of convection bound systems and the high plate height and bandspreading characteristics of diffusion bound systems. This can be accomplished by forcing chromatography fluids through a matrix having a set of larger pores, such as are defined by the interstices among a bed of particles, and which determine pressure drops and fluid flow velocities through the bed, and a set of pores of smaller diameter, e.g., anisotropic throughpores. The smaller pores permeate the individual particles and serve to deliver chromatography fluids by convection to surface regions within the particle interactive with the solutes in the chromatography fluid.

DEPR:
From the foregoing description many of the basic engineering goals to be pursued in the fabrication of matrix materials suitable for the practice of [illegible] chromatography will be apparent to those skilled in the art. Thus, what is needed in practice perfusion chromatography is a matrix which will not crush under pressure having a bimodal or preferably **multimodal** pore structure and as large a surface area per unit volume as possible. The first and second pore sets which give the material its bimodal flow properties

must have mean
diameters relative to each other so as to permit convective flow through both
sets of pores at high V.sub.beds. The provision of subpores in the matrix is
not required to conduct perfusion chromatography but is preferred because of
the inherent increase in surface area per unit volume of matrix material such a
construction provides.

DEPR:
In contrast to the PL 4,000 material, which, with respect to its pore
structure, is **multimodal,** a more ideal perfusive particle might comprise a
plurality of sets of throughpores and subpores of differing mean diameters. A
bimodal pore size distribution can be achieved in such particle by mixing equal
ratios of particles having two discrete pore sizes or by engineering this
feature at the polymerization stage. Ideally, mean diameter ratio between
throughpore subsets would be less than 10, the mean diameter ratio between the
smallest throughpore sets and the subpores would be less than 20, and the mean
diameter ratio between the first pore set, i.e., the interstices among the
particles making up the matrix, and the largest throughpore subset would be
less than 70, and preferably less than 50. A **multimodal** material might be
produced by agglomerating 500 .ANG. porons to form approximately 1 .mu.m
clusters, which in turn are agglomerated to form 10 .mu.m aggregates, which in
turn may be aggregated to form 100 .mu.m particles. In such a [illegible]
.mu.m clusters would have interstices of a mean diameter [illegible]
a few hundred .ANG.. These would define the [illegible]
surface area. Diffusive transport within these pores would rarely have to
exceed a distance of 0.5 .mu.m or 5,000 .ANG.. Interstices among the 1 .mu.m
clusters making up the 10 .mu.m aggregates would permit convective flow to feed

the diffusive pores. These would be on the order of 0.3 .mu.m in diameter.
These throughpores, in turn would be fed by larger pores defined by
intersticies among the 10 .mu.m particles making up the 100 .mu.m particles.
These would have a mean diameter on the order of 35 .mu.m.

CCOR:
210/198.2

DOCUMENT-IDENTIFIER: US 5104530 A
TITLE: Chromatography column with carbonaceous adsorbents from pyrolyzed
polysulfonated polymers

ABPL:
Carbonaceous adsorbent particles having **multimodal** pore size, including
micropores and macropores, with improved adsorptive and separative properties,
are prepared by partial pyrolysis of polysulfonated macroporous precursor
resins, said resins being in turn derived from macroporous poly(vinylaromatic)
resins. The particles may be further treated by activating with reactive gases
or by functionalization.

BSPR:
British Patent No 1,525,420, in a broad description of method for rendering
infusible various porous high molecular weight compounds (including macroporous
resins), and then calcining them, relates techniques for polysulfonation
earlier described by Corte et al. among those suitable for creating
infusibility. No characterization data are given for the polymer prior to
calcination. Preferred infusibility reactants are sulfur trioxide, sulfuric
acid, or chlorosulfonic acid. This reference discloses pyrolysis of
macroporous resins treated with 15% fuming sulfuric acid and pyrolyzed, and
describes an experimental method for determining the porosity of the pyrolyzed
material down to 1.5 nm. The results described in the [illegible]
show the absence of any porosity development [illegible], but
multimodal
porosity is not taught. In contrast, Neely in the cited references fully shows
the development of microporosity for monosulfonated macroporous resins.
Further, the British patent is silent about the processing advantages observed

in pyrolysis of polysulfonated resins.

CLPR:
1. A chromatographic column packed with the carbonaceous adsorbent particles which comprise the product of controlled pyrolysis of a polysulfonated macroporous crosslinked, vinylaromatic polymer, the particles having **multimodal** pore-size distribution and a minimum micropore volume of about 0.02 cm.sup.3 /g.

CLPR:
4. A chromatographic column packed with the carbonaceous adsorbent particles which comprise the product of controlled pyrolysis of a polysulfonated macroporous crosslinked, vinylaromatic polymer, the particles having **multimodal** pore-size distribution and a minimum micropore volume of about 0.02 cm.sup.3 /g, wherein the particles are treated, subsequent to pyrolysis, with adsorbable reactive agent.

CCOR:
210/198.2

DOCUMENT-IDENTIFIER: US 5071547 A
TITLE: Column chromatographic column apparatus with switching capability

DEPR:
The fluid conduit assembly or means is comprised of conduit piping connecting
the dual columns, detector, multi-valve arrangement in a similar manner to that
of FIG. 2 with three exceptions. First, the fluid conduit assembly in addition
to conduits 11A through 11L as in FIG. 2, also has conduits 11X through 11Z and
11Z'. Second, there are two valves present in what conceptually can be
referred to as each valve set 12A and 12B of FIG. 2. The third exception is
the presence of an additional valve 42 that isolates the detector from the
pressure of the DCC Apparatus Although valves 12iv and 12v can be indicated as
being inthe valve set 12A of FIG. 2 and valves 12vi and 12vii can be indicated
as being in the valve set 12B of FIG. 2, these valves may exist with
independent identity from these valve sets. In other words these valves may be
just aplurality of valves without valves 12iv and 12v as well as the two valves
12vi and 12vii perform the same function as valves sets 12A and 12B,
respectively. Valve 42 is any multi-port and **multimodal** valve known to those
skilled in the art as are valves 12iv through 12vii, and valve 42 can be
considered a part of the multi-valve arrangement 12. As with the DCC Apparatus
of FIG. 2, that of FIG. 3 has each valve with an actuating [illegible]
controller 13. So valve 42 is also [illegible]
actuating connection 14A as valves 12iv-12vii and 42 are connected by
connections 14J, 14K, 14L, 14M, and 14N, respectively. Valves 12iv and 12v are
connected together for pressurized fluid passage between them by conduit 11X,

and in a similar manner valves 12vi and 12vii are connected by conduit 11Y.

CCOR:
210/198.2

DOCUMENT-IDENTIFIER: US 5019270 A
TITLE: Perfusive chromatography

DEPR:
Broadly, in accordance with the invention, perfusion chromatography is practiced by passing fluids at velocities above a threshold level through a specially designed matrix characterized by a geometry which is bimodal or **multimodal** with respect to its porosity. Perhaps the most fundamental observation relevant to the new procedure is that it is possible to avoid both the loss of capacity characteristic of convection bound systems and the high plate height and bandspreading characteristics of diffusion bound systems. This can be accomplished by forcing chromatography fluids through a matrix having a set of larger pores, such as are defined by the interstices among a bed of particles, and which determine pressure drops and fluid flow velocities through the bed, and a set of pores of smaller diameter, e.g., anisotropic throughpores. The smaller pores permeate the individual particles and serve to deliver chromatography fluids by convection to surface regions within the particle interactive with the solutes in the chromatography fluid.

DEPR:
From the foregoing description many of the basic engineering goals to be pursued in the fabrication of matrix materials suitable for the practice of [illegible] chromatography will be apparent to those skilled in the art. Thus, a matrix material to practice perfusion chromatography is a matrix which will not crush under pressure having a bimodal or preferably **multimodal** pore structure and as large a surface area per unit volume as possible. The first and second pore sets which give the material its bimodal flow properties

must have mean
diameters relative to each other so as to permit convective flow through both
sets of pores at high V.sub.beds. The provision of subpores in the matrix is
not required to conduct perfusion chromatography but is preferred because of
the inherent increase in surface area per unit volume of matrix material such a
construction provides.

DEPR:
In contrast to the PL 4,000 material, which, with respect to its pore
structure, is **multimodal,** a more ideal perfusive particle might comprise a
plurality of sets of throughpores and subpores of differing mean diameters. A
bimodal pore size distribution can be achieved in such particle by mixing equal
ratios of particles having two discrete pore sizes or by engineering this
feature at the polymerization stage. Ideally, mean diameter ratio between
throughpore subsets would be less than 10, the mean diameter ratio between the
smallest throughpore sets and the subpores would be less than 20, and the mean
diameter ratio between the first pore set, i.e., the intersticies among the
particles making up the matrix, and the largest throughpore subset would be
less than 70, and preferably less than 50. A **multimodal** material might be
produced by agglomerating 500 .ANG. porons to form approximately 1 .mu.m
clusters, which in turn are agglomerated to form 10 .mu.m aggregates, which in
turn may be aggregated to form 100 .mu.m particles. In such a [illegible]
.mu. m clusters would have intersticies of a mean diameter in [illegible]
a few hundred .ANG.. Those would [illegible] a very high
surface area. Diffusive transport within these pores would rarely have to
exceed a distance of 0.5 .mu.m or 5,000 .ANG.. Intersticies among the 1 .mu.m
clusters making up the 10 .mu.m aggregates would permit convective flow to feed

the diffusive pores. These would be on the order of 0.3 .mu.m in diameter.
These throughpores, in turn would be fed by larger pores defined by
intersticies among the 10 .mu.m particles making up the 100 .mu.m particles.
These would have a mean diameter on the order of 35 .mu.m.

CCXR:
210/198.2

DOCUMENT-IDENTIFIER: US 5833861 A
TITLE: Perfusive chromatography

DEPR:
Broadly, in accordance with the invention, perfusion chromatography is practiced by passing fluids at velocities above a threshold level through a specially designed matrix characterized by a geometry which is bimodal or **multimodal** with respect to its porosity. Perhaps the most fundamental observation relevant to the new procedure is that it is possible to avoid both the loss of capacity characteristic of convection bound systems and the high plate height and bandspreading characteristics of diffusion bound systems. This can be accomplished by forcing chromatography fluids through a matrix having a set of larger pores, such as are defined by the interstices among a bed of particles, and which determine pressure drops and fluid flow velocities through the bed, and a set of pores of smaller diameter, e.g., anisotropic throughpores. The smaller pores permeate the individual particles and serve to deliver chromatography fluids by convection to surface regions within the particle interactive with the solutes in the chromatography fluid.

DEPR:
From the foregoing description many of the basic engineering goals to be aimed at in the fabrication of matrix materials suitable for the practice of useful chromatography will be apparent to those skilled in the art. Thus, a matrix suitable for practice perfusion chromatography is a matrix which will not crush under pressure having a bimodal or preferably **multimodal** pore structure and as large a surface area per unit volume as possible. The first and second pore sets which give the material its bimodal flow properties

must have mean
diameters relative to each other so as to permit convective flow through both
sets of pores at high V.sub.beds. The provision of subpores in the matrix is
not required to conduct perfusion chromatography but is preferred because of
the inherent increase in surface area per unit volume of matrix material such a
construction provides.

DEPR:
In contrast to the PL 4,000 material, which, with respect to its pore
structure, is **multimodal,** a more ideal perfusive particle might comprise a
plurality of sets of throughpores and subpores of differing mean diameters. A
bimodal pore size distribution can be achieved in such particle by mixing equal
ratios of particles having two discrete pore sizes or by engineering this
feature at the polymerization stage. Ideally, mean diameter ratio between
throughpore subsets would be less than 10, the mean diameter ratio between the
smallest throughpore sets and the subpores would be less than 20, and the mean
diameter ratio between the first pore set, i.e., the intersticies among the
particles making up the matrix, and the largest throughpore subset would be
less than 70, and preferably less than 50. A **multimodal** material might be
produced by agglomerating 500 .ANG. porons to form approximately 1 .mu.m
clusters, which in turn are agglomerated to form 10 .mu.m aggregates, which in
turn may be aggregated to form 100 .mu.m particles. In such a [illegible]
.mu.m clusters would have interstitles of a mean diameter in the [illegible]
few hundred .ANG.. [illegible]
surface area. Diffusive transport within these pores would rarely have to
exceed a distance of 0.5 .mu.m or 5,000 .ANG.. Intersticies among the 1 .mu.m
clusters making up the 10 .mu.m aggregates would permit convective flow to feed

the diffusive pores. These would be on the order of 0.3 .mu.m in diameter.
These throughpores, in turn would be fed by larger pores defined by
intersticies among the 10 .mu.m particles making up the 100 .mu.m particles.
These would have a mean diameter on the order of 35 .mu.m.

CCXR:
210/198.2

DOCUMENT-IDENTIFIER: US 5605623 A
TITLE: Perfusive chromatography

DEPR:
Broadly, in accordance with the invention, perfusion chromatography is practiced by passing fluids at velocities above a threshold level through a specially designed matrix characterized by a geometry which is bimodal or **multimodal** with respect to its porosity. Perhaps the most fundamental observation relevant to the new procedure is that it is possible to avoid both the loss of capacity characteristic of convection bound systems and the high plate height and bandspreading characteristics of diffusion bound systems. This can be accomplished by forcing chromatography fluids through a matrix having a set of larger pores, such as are defined by the interstices among a bed of particles, and which determine pressure drops and fluid flow velocities through the bed, and a set of pores of smaller diameter, e.g., anisotropic throughpores. The smaller pores permeate the individual particles and serve to deliver chromatography fluids by convection to surface regions within the particle interactive with the solutes in the chromatography fluid.

DEPR:
From the foregoing description many of the basic engineering goals to be pursued in the fabrication of matrix materials suitable for the practice of perfusion chromatography will be apparent to those skilled in the art. Thus, what is needed to practice perfusion chromatography is a matrix which will not strain under pressure having a bimodal or preferably **multimodal** pore structure and as large a surface area per unit volume as possible. The first and second pore sets which give the material its bimodal flow properties

must have mean
diameters relative to each other so as to permit convective flow through both
sets of pores at high V.sub.bods. The provision of subpores in the matrix is
not required to conduct perfusion chromatography but is preferred because of
the inherent increase in surface area per unit volume of matrix material such a
construction provides.

DEPR:
In contrast to the PL 4,000 material, which, with respect to its pore
structure, is **multimodal,** a more ideal perfusive particle might comprise a
plurality of sets of throughpores and subpores of differing mean diameters. A
bimodal pore size distribution can be achieved in such particle by mixing equal
ratios of particles having two discrete pore sizes or by engineering this
feature at the polymerization stage. Ideally, mean diameter ratio between
throughpore subsets would be less than 10, the mean diameter ratio between the
smallest throughpore sets and the subpores would be less than 10, and the mean
diameter ratio between the first pore set, i.e., the interstices among the
particles making UP the matrix, and the largest throughpore subset would be
less than 70, and preferably less than 50. A **multimodal** material might be
produced by agglomerating 500 .ANG. porons to form approximately 1 .mu.m
clusters, which in turn are agglomerated to form 10 .mu.m aggregates, which in
turn may be aggregated to form 100 .mu.m particles. In such a [illegible]
.mu.m clusters would have interstices of a mean diameter in the [illegible]
few hundred .ANG.. These would define the [illegible]
surface area. Diffusive transport within these pores would rarely have to
exceed a distance of 0.5 .mu.m or 5,000 .ANG.. Interstices among the 1 .mu.m
clusters making up the 10 .mu.m aggregates would permit convective flow to feed

the diffusive pores. These would be on the order of 0.3 .mu.m in diameter.
These throughpores, in turn would be fed by larger pores defined by
intersticies among the 10 .mu.m particles making up the 100 .mu.m particles.
These would have a mean diameter on the order of 35 .mu.m.

CLPR:
8. The chromatography system of claim 7 wherein the packed particles define a
bimodal or **multimodal** pore structure.

CCOR:
210/198.2

DOCUMENT-IDENTIFIER: US 5552041 A
TITLE: Perfusive chromatography

DEPR:
Broadly, in accordance with the invention, perfusion chromatography is practiced by passing fluids at velocities above a threshold level through a specially designed matrix characterized by a geometry which is bimodal or **multimodal** with respect to its porosity. Perhaps the most fundamental observation relevant to the new procedure is that it is possible to avoid both the loss of capacity characteristic of convection bound systems and the high plate height and bandspreading characteristics of diffusion bound systems. This can be accomplished by forcing chromatography fluids through a matrix having a set of larger pores, such as are defined by the interstices among a bed of particles, and which determine pressure drops and fluid flow velocities through the bed, and a set of pores of smaller diameter, e.g., anisotropic throughpores. The smaller pores permeate the individual particles and serve to deliver chromatography fluids by convection to surface regions within the particle interactive with the solutes in the chromatography fluid.

DEPR:
From the foregoing description many of the basic engineering goals to be [illegible] in the fabrication of matrix materials suitable for the practice of [illegible] chromatography will be apparent to those skilled in the art. Thus, [illegible] for practice perfusion chromatography is a matrix which will not crush under pressure having a bimodal or preferably **multimodal** pore structure and as large a surface area per unit volume as possible. The first and second pore sets which give the material its bimodal flow properties

must have mean
diameters relative to each other so as to permit convective flow through both
sets of pores at high V.sub.beds. The provision of subpores in the matrix is
not required to conduct perfusion chromatography but is preferred because of
the inherent increase in surface area per unit volume of matrix material such a
construction provides.

DEPR:
In contrast to the PL 4,000 material, which, with respect to its pore
structure, is **multimodal,** a more ideal perfusive particle might comprise a
plurality of sets of throughpores and subpores of differing mean diameters. A
bimodal pore size distribution can be achieved in such particle by mixing equal
ratios of particles having two discrete pore sizes or by engineering this
feature at the polymerization stage. Ideally, mean diameter ratio between
throughpore subsets would be less than 10, the mean diameter ratio between the
smallest throughpore sets and the subpores would be less than 20, and the mean
diameter ratio between the first pore set, i.e., the intersticies among the
particles making up the matrix, and the largest throughpore subset would be
less than 70, and preferably less than 50. A **multimodal** material might be
produced by agglomerating 500 .ANG. porons to form approximately 1 .mu.m
clusters, which in turn are agglomerated to form 10 .mu.m aggregates, which in
turn may be aggregated to form 100 .mu.m particles. In such a [illegible] the 1
.mu.m clusters would have interstices of a mean diameter in the [illegible]
few hundred .ANG.. These would define the [illegible] very high
surface area. Diffusive transport within these pores would rarely have to
exceed a distance of 0.5 .mu.m or 5,000 .ANG.. Interstices among the 1 .mu.m
clusters making up the 10 .mu.m aggregates would permit convective flow to feed

the diffusive pores. These would be on the order of 0.3 .mu.m in diameter.
These throughpores, in turn would be fed by larger pores defined by
intersticies among the 10 .mu.m particles making up the 100 .mu.m particles.
These would have a mean diameter on the order of 35 .mu.m.

CCOR:
210/198.2

DOCUMENT-IDENTIFIER: US 5540834 A
TITLE: Synthesis of porous inorganic particles by polymerization-induced colloid aggregation (PICA)

DEPR:
The specific surface area and porosity of the sintered particles measured by nitrogen adsorption were 13 m.sup.2 /g and 29%, respectively, which are in reasonable agreement with close-packed, dense ZrO.sub.2 spheres with nonuniform particle size. The pore size distributions (psd) obtained by nitrogen adsorption and desorption, and mercury porosimetry (intrusion) are displayed in FIGS. 4A, 4B and FIG. 5, respectively. From N.sub.2 adsorption, the psd was determined to be rather narrow with a maximum near 400 .ANG. and a small contribution of pores larger than 500 .ANG. and smaller than 100 .ANG.. From N.sub.2 desorption, the psd was determined to be **multimodal** with nearly all pores between 100 .ANG. and 200 .ANG. in diameter and some pores below 50 .ANG.. This discrepancy is mainly due to pore blocking or network effects, whereby desorption from a pore in a network is influenced by the state of the neighboring pores. Nitrogen adsorption probes the main channel size and can be considered free of pore blocking effects, while nitrogen desorption shows a disproportionately large amount of small pores due to "bottle necks." The psd obtained from mercury porosimetry (intrusion) are also influenced by pore interconnections; as shown in FIG. 5, it is broad with pores between approximately 125 .ANG. and 500 .ANG. in diameter and a maximum near 200 .ANG., in reasonable agreement with the psd from N.sub.2 desorption.

DEPR:
The pore-size distributions (psd's) after sintering are shown in

FIG. 19. All samples exhibited **multimodal** psd's with pore diameters ranging between 100 .ANG. and 450 .ANG.. The sample synthesized at pH 1.2 contained some hollow particles but its psd seemed qualitatively similar to those of samples synthesized at higher pH (non-hollow particles). This is because N.sub.2 adsorption only probes the pores within the ZrO.sub.2 shells and not the large voids they encompass. Surface areas and porosities (.epsilon..sub.particle) for these samples are listed in Table 3. Note that there is qualitative agreement with FIG. 12E, but quantitative agreement is not expected since condensing N.sub.2 cannot be used to distinguish between hollow cores and interstitial volume between aggregates. The N.sub.2 psd's show that the higher the pH, the greater contribution of small pores to the total porosity.

CCOR:
210/198.2

DOCUMENT-IDENTIFIER: US 5522994 A
TITLE: Single column chromatographic determination of small molecules in
mixtures with large molecules

CCXR:
210/198.2

ORPL:
Little, "Sequential **Multimodal** Elution for Pseudomultidimensional Liquid
Chromatography on a Single Column," Anal. Chem., 63 (1991) pp. 33-34.

DOCUMENT-IDENTIFIER: US 5431807 A
TITLE: **Multimodal** chromatographic separation media and process for using same

TTL:
Multimodal chromatographic separation media and process for using same

ABPL:
A process for carrying out in a consecutive fashion different modes of
chromatographic separation in a liquid chromatography column using a single
separation medium is disclosed. Separation media for use in such **multimodal**
separations are also disclosed.

BSPR:
It may be possible to use combinations of different separation media in
different columns for **multimodal** separations. An example of this multiple
column bimodal separation was described recently by Wheatley J. B., J.
Chromatogr., 603 (1992) 273. The bimodal separation of small molecules in one
column packed with one separation medium and based on sequential **multimodal**
elution was described by Little E. L., Jeansonne M. S., Foley J. P.; Anal
Chem., 63, 1991, 33. They combined ion-exchange and reversed phase
chromatography for the separation of a complex sample containing two groups of
compounds: charged and non-polar. The use of two different gradients, i.e. a
pH gradient and a methanol gradient, resulted in the separation of the charged
[illegible] followed by the separation of the neutral molecules after
switching to the second mobile phase. This approach makes use of imperfect
surface functionalization of porous silica beads which contained C.sub.1,
C.sub.8 or C.sub.18 groups together with the original acidic surface silanol
groups. Similarly, the DIONEX OmniPack PAX-500 column is packed

with
non-porous poly[styrene-divinylbenzene] beads coated on the bead surface with
attached ion-exchange latex particles (as described by the DIONEX booklet).
Here again, the coating of the bead surface is imperfect and it is the
non-covered hydrophobic areas of the original non-porous beads that are used
for separation in the second mode. This approach excludes combinations not
involving the reversed phase mode (the original ST-DVB surface remains
non-polar even after attachment of latex particles) as well as any size
exclusion separation.

BSPR:
This **multimodal** separation process is able to achieve separation in a single
column in a consecutive operation because of the properties of the separation
medium. The separation medium generally comprises a porous material which has
been pretreated so that it has at least two different types of surface groups
which have different functionalities. These different surface groups are
disposed in different size range pores within the porous material. Pore size
as used herein can mean a single measured average size, for example, 25 nm, but
in most cases it means a particular range of sizes, for example, 50-500 nm. An
example of such a porous material of the present invention is one wherein there
are hydrophilic surface groups in pores having a size of from about 5-25 nm and
hydrophobic surface groups in pores ranging in a size of from [illegible] nm.
Another example is a material having hydrophilic groups in pores [illegible] nm in
size and hydrophobic groups in pores above [illegible]. [illegible]
the different functionalities of the surface groups, molecules that have
affinities to such different surface groups may be separated during different
modes of separation, which may be carried out in a consecutive fashion. As

used herein, different molecules means molecules of different sizes, different
chemical affinities, different structures, compositions, polarities,
chiralities, activities, etc.

DRPR:
The key in selecting a mobile phase for a **multimodal** separation is the
consecutive use of mobile phases, in each individual mode, that do not
interfere with the absorption of compounds to be separated in any of the
subsequent modes. Otherwise, the separation will not be **multimodal** and one
group of compounds will leave the column without any separation, as documented
in Example 6 and FIG. 7. Under this assumption, even a mobile phase for
trimodal separation is easily designed by a person skilled in the art of liquid
chromatography.

DRPR:
Reaction Schemes 1-4 not only describe the particular sets of reactions leading
to **multimodal** separation media but they also show the concepts of making such
media in general. The starting polymer must be porous with relatively broad
pore size distribution and possess reactive groups on the surface of the pores.
Typically, the pore-size selectivity of the modification reactions are
controlled by the molecular weight of the catalyst or reagent used in the
particular modifying reaction and by the solvent. The number of modes
accommodated in a separation medium is theoretically not limited but
practically will rarely exceed three. The most important part in designing the
reaction strategy for preparation of a **multimodal** [illegible]
the path. The product of a given reaction affecting pores of a given size
should not affect the groups already built up in the previous reaction step
within pores of a different size.

DRPR:
The **multimodal** separation process of the present invention may even use very tiny differences between the separation modes as is the case with reversed phase and hydrophobic interaction chromatography. The separation medium can be prepared by a set of reactions shown in Reaction Scheme 5.

CLPR:
1. A **multimodal** separation medium for use in liquid chromatography comprising a porous separation medium having at least two different pore size ranges with each pore size range containing a different surface group, having a different functionality compared to the surface groups in the other pore size range, said porous separation medium being capable of separating molecules in a sample added to a chromatography column containing said separation medium during different modes of separation which are carried out in a consecutive fashion using a single separation medium.

CLPR:
2. A **multimodal** separation medium for use in chromatography comprising a porous separation medium having at least two ranges of pore size, with each range of pore sizes having surface groups of a chemical composition different from that of other pore size ranges.

CCOR:
210/198.2

OTPL:
Little, "Sequential **Multimodal** Elution for Pseudomultidimensional Liquid [illegible] on a Single Column," Anal. Chem., 63 (1991) pp. 33-44.

DOCUMENT-IDENTIFIER: US 5384042 A
TITLE: Perfusive chromatography

DEPR:
Broadly, in accordance with the invention, perfusion chromatography is practiced by passing fluids at velocities above a threshold level through a specially designed matrix characterized by a geometry which is bimodal or **multimodal** with respect to its porosity. Perhaps the most fundamental observation relevant to the new procedure is that it is possible to avoid both the loss of capacity characteristic of convection bound systems and the high plate height and bandspreading characteristics of diffusion bound systems. This can be accomplished by forcing chromatography fluids through a matrix having a set of larger pores, such as are defined by the intersticies among a bed of particles, and which determine pressure drops and fluid flow velocities through the bed, and a set of pores of smaller diameter, e.g., anisotropic throughpores. The smaller pores permeate the individual particles and serve to deliver chromatography fluids by convection to surface regions within the particle interactive with the solutes in the chromatography fluid.

DEPR:
From the foregoing description many of the basic engineering goals to be pursued in the fabrication of matrix materials suitable for the practice of perfusion chromatography will be apparent to those skilled in the art. Thus, a matrix suitable for practice perfusion chromatography is a matrix which will not crush under pressure having a bimodal or preferably **multimodal** pore structure and as large a surface area per unit volume as possible. The first and second pore sets which give the material its bimodal flow properties

must have mean
diameters relative to each other so as to permit convective flow through both
sets of pores at high V.sub.beds. The provision of subpores in the matrix is
not required to conduct perfusion chromatography but is preferred because of
the inherent increase in surface area per unit volume of matrix material such a
construction provides.

DEPR:
In contrast to the PL 4,000 material, which, with respect to its pore
structure, is **multimodal,** a more ideal perfusive particle might comprise a
plurality of sets of throughpores and subpores of differing mean diameters. A
bimodal pore size distribution can be achieved in such particle by mixing equal
ratios of particles having two discrete pore sizes or by engineering this
feature at the polymerization stage. Ideally, mean diameter ratio between
throughpore subsets would be less than 10, the mean diameter ratio between the
smallest throughpore sets and the subpores would be less than 20, and the mean
diameter ratio between the first pore set, i.e., the intersticies among the
particles making up the matrix, and the largest throughpore subset would be
less than 70, and preferably less than 50. A **multimodal** material might be
produced by agglomerating 500 .ANG. porons to form approximately 1 .mu.m
clusters, which in turn are agglomerated to form 10 .mu.m aggregates, which in
turn may be aggregated to form 100 .mu.m particles. In such a [illegible]
.mu.m clusters would have intersticies of a mean diameter in [illegible]
a few hundred .ANG.. These would define the [illegible]
surface area. Diffusive transport within these pores would rarely have to
exceed a distance of 0.5 .mu.m or 5,000 .ANG.. Intersticies among the 1 .mu.m
clusters making up the 10 .mu.m aggregates would permit convective flow to feed

the diffusive pores. These would be on the order of 0.3 .mu.m in diameter.
These throughpores, in turn would be fed by larger pores defined by
intersticies among the 10 .mu.m particles making up the 100 .mu.m particles.
These would have a mean diameter on the order of 35 .mu.m.

CLPF:
22. A matrix for conducting high efficiency adsorption chromatography of
biological molecules, the matrix comprising a packed bed of rigid, polystyrene
divinylbenzene particles having a mean diameter between 10 and 20 micrometers,
defining a bimodal or **multimodal** pore structure and comprising chemically
active regions linked to the surface of the particles for reversibly sorbing
biological molecules, said matrix being characterized in that

CLPV:
a packed bed of rigid particles comprising an inorganic material, the particles
having a mean diameter within the range of 10 micrometers to 100 micrometers
and defining a bimodal or **multimodal** pore structure, one set of pores being
particle transecting throughpores having a mean diameter greater than at least
4000 .ANG., another set of pores being subpores in fluid communication with the
throughpores, and, disposed within at least said subpores, reactive groups
comprising one of anionic sulfonate groups, cationic quaternary ammonium
groups, immunoglobulines, or hydrocarbons, the ratio of the means diameter of
the particles to the mean diameter of a throughpores passing through the
particle being sufficient to permit convective transport of biological
molecules into the throughpores [illegible] chromatographic
separation so that, at flow rates greater than 100 cm./hr, the rate of
biological molecule transport into the throughpores is dependent on the
velocity of liquid passing through the bed, and sorption capacity remains

substantially constant over a range of flowrates.

CCOR:
210/198.2

DOCUMENT-IDENTIFIER: US 5316680 A
TITLE: **Multimodal** chromatographic separation media and process for using same

TTL:
Multimodal chromatographic separation media and process for using same

ABPL:
A process for carrying out in a consecutive fashion different modes of chromatographic separation in a liquid chromatography column using a single separation medium is disclosed. Separation media for use in such **multimodal** separations are also disclosed.

BSPR:
It may be possible to use combinations of different separation media in different columns for **multimodal** separations. An example of this multiple column bimodal separation was described recently by Wheatley J. B., J. Chromatogr., 603 (1992) 273. The bimodal separation of small molecules in one column packed with one separation medium and based on sequential **multimodal** elution was described by Little E. L., Jeansonne M. S., Foley J. P.; Anal Chem., 63, 1991, 93. They combined ion-exchange and reversed phase chromatography for the separation of a complex sample containing two groups of compounds: charged and non-polar. The use of two different gradients, i.e. a [illegible] gradient and a methanol gradient, resulted in the separation of the charged [illegible], followed by the separation of the neutral molecules after switching to the second mobile phase. This approach makes use of imperfect surface functionalization of porous silica beads which contained C.sub.1, C.sub.8 or C.sub.18 groups together with the original acidic surface silanol groups. Similarly, the DIONEX OmniPack PAX-500 column is packed

with
non-porous poly[styrene-divinylbenzene] beads coated on the bead surface with
attached ion-exchange latex particles (as described by the DIONEX booklet).
Here again, the coating of the bead surface is imperfect and it is the
non-covered hydrophobic areas of the original non-porous beads that are used
for separation in the second mode. This approach excludes combinations not
involving the reversed phase mode (the original ST-DVB surface remains
non-polar even after attachment of latex particles) as well as any size
exclusion separation.

BSPR:
This **multimodal** separation process is able to achieve separation in a single
column in a consecutive operation because of the properties of the separation
medium. The separation medium generally comprises a porous material which has
been pretreated so that it has at least two different types of surface groups
which have different functionalities. These different surface groups are
disposed in different size range pores within the porous material. Pore size
as used herein can mean a single measured average size, for example, 25 nm, but
in most cases it means a particular range of sizes, for example, 50-500 nm. An
example of such a porous material of the present invention is one wherein there
are hydrophilic surface groups in pores having a size of from about 5-25 nm and
hydrophobic surface groups in pores ranging in a size of from about 30-50 nm.
Another example is a material having hydrophilic groups in pores [illegible]
size and hydrophobic groups in pores [illegible]
the different functionalities of the surface groups, molecules that have
affinities to such different surface groups may be separated during different
modes of separation, which may be carried out in a consecutive fashion. As

used herein, different molecules means molecules of different sizes, different
chemical affinities, different structures, compositions, polarities,
chiralities, activities, etc.

DEPR:
The key in selecting a mobile phase for a **multimodal** separation is the
consecutive use of mobile phases, in each individual mode, that do not
interfere with the absorption of compounds to be separated in any of the
subsequent modes. Otherwise, the separation will not be **multimodal** and one
group of compounds will leave the column without any separation, as documented
in Example 6 and FIG. 7. Under this assumption, even a mobile phase for
trimodal separation is easily designed by a person skilled in the art of liquid
chromatography.

DEPR:
Reaction Schemes 1-4 not only describe the particular sets of reactions leading
to **multimodal** separation media but they also show the concepts of making such
media in general. The starting polymer must be porous with relatively broad
pore size distribution and possess reactive groups on the surface of the pores.
Typically, the pore-size selectivity of the modification reactions are
controlled by the molecular weight of the catalyst or reagent used in the
particular modifying reaction and by the solvent. The number of modes
accommodated in a separation medium is theoretically not limited but
practically will rarely exceed three. The most important part in designing a
reaction strategy for preparation of a **multimodal** medium is to make the
right choice at
the path. The product of a given reaction affecting pores of a given size
should not affect the groups already built up in the previous reaction step
within pores of a different size.

DEPR:
The **multimodal** separation process of the present invention may even use very
tiny differences between the separation modes as is the case with reversed
phase and hydrophobic interaction chromatography. The separation medium can be
prepared by a set of reactions shown in Reaction Scheme 5.

CCXR:
210/198.2

ORPL:
Little, "Sequential **Multimodal** Elution for Pseudomultidimensional Liquid
Chromatography on a Single Column," Anal. Chem., 63, (1991) pp. 33-44.

DOCUMENT-IDENTIFIER: US 5228989 A
TITLE: Perfusive chromatography

DEPR:
Broadly, in accordance with the invention, perfusion chromatography is practiced by passing fluids at velocities above a threshold level through a specially designed matrix characterized by a geometry which is bimodal or **multimodal** with respect to its porosity. Perhaps the most fundamental observation relevant to the new procedure is that it is possible to avoid both the loss of capacity characteristic of convection bound systems and the high plate height and bandspreading characteristics of diffusion bound systems. This can be accomplished by forcing chromatography fluids through a matrix having a set of larger pores, such as are defined by the interstices among a bed of particles, and which determine pressure drops and fluid flow velocities through the bed, and a set of pores of smaller diameter, e.g., anisotropic throughpores. The smaller pores permeate the individual particles and serve to deliver chromatography fluids by convection to surface regions within the particle interactive with the solutes in the chromatography fluid.

DEPR:
From the foregoing description many of the basic engineering goals to be pursued in the fabrication of matrix materials suitable for the practice of perfusion chromatography will be apparent to those skilled in the art. Thus, what is needed to practice perfusion chromatography is a matrix which will not crush under pressure having a bimodal or preferably **multimodal** pore structure and as large a surface area per unit volume as possible. The first and second pore sets which give the material its bimodal flow properties

must have mean
diameters relative to each other so as to permit convective flow through both
sets of pores at high V.sub.beds. The provision of subpores in the matrix is
not required to conduct perfusion chromatography but is preferred because of
the inherent increase in surface area per unit volume of matrix material such a
construction provides.

DEPR:
In contrast to the PL 4,000 material, which, with respect to its pore
structure, is **multimodal,** a more ideal perfusive particle might comprise a
plurality of sets of throughpores and subpores of differing mean diameters. A
bimodal pore size distribution can be achieved in such particle by mixing equal
ratios of particles having two discrete pore sizes or by engineering this
feature at the polymerization stage. Ideally, mean diameter ratio between
throughpore subsets would be less than 10, the mean diameter ratio between the
smallest throughpore sets and the subpores would be less than 20, and the mean
diameter ratio between the first pore set, i.e., the intersticies among the
particles making up the matrix, and the largest throughpore subset would be
less than 70, and preferably less than 50. A **multimodal** material might be
produced by agglomerating 500 .ANG. porons to form approximately 1 .mu.m
clusters, which in turn are agglomerated to form 10 .mu.m aggregates, which in
turn may be aggregated to form 100 .mu.m particles. In such a [illegible]
.mu.m clusters would have intersticies of a mean diameter in [illegible]
a few hundred .ANG.. Those would define the [illegible]
surface area. Diffusive transport within these pores would rarely have to
exceed a distance of 0.5 .mu.m or 5,000 .ANG.. Intersticies among the 1 .mu.m
clusters making up the 10 .mu.m aggregates would permit convective flow to feed

the diffusive pores. These would be on the order of 0.3 .mu.m in diameter.
These throughpores, in turn would be fed by larger pores defined by
intersticies among the 10 .mu.m particles making up the 100 .mu.m particles.
These would have a mean diameter on the order of 35 .mu.m.

CCOR:
210/198.2

DOCUMENT-IDENTIFIER: US 5104530 A
TITLE: Chromatography column with carbonaceous adsorbents from pyrolyzed
polysulfonated polymers

ABPL:
Carbonaceous adsorbent particles having **multimodal** pore size, including
micropores and macropores, with improved adsorptive and separative properties,
are prepared by partial pyrolysis of polysulfonated macroporous precursor
resins, said resins being in turn derived from macroporous poly(vinylaromatic)
resins. The particles may be further treated by activating with reactive gases
or by functionalization.

BSPR:
British Patent No 1,525,420, in a broad description of method for rendering
infusible various porous high molecular weight compounds (including macroporous
resins), and then calcining them, relates techniques for polysulfonation
earlier described by Corte et al. among those suitable for creating
infusibility. No characterization data are given for the polymer prior to
calcination. Preferred infusibility reactants are sulfur trioxide, sulfuric
acid, or chlorosulfonic acid. This reference discloses pyrolysis of
macroporous resins treated with 15% fuming sulfuric acid and pyrolyzed, and
describes an experimental method for determining the porosity of [illegible]
material down to 2-5 nm. The results described in the tables of [illegible]
show the absence of any porosity development [illegible], but
multimodal
porosity is not taught. In contrast, Neely in the cited references fully shows
the development of microporosity for monosulfonated macroporous resins.
Further, the British patent is silent about the processing advantages observed

in pyrolysis of polysulfonated resins.

CLPR:
1. A chromatographic column packed with the carbonaceous adsorbent particles which comprise the product of controlled pyrolysis of a polysulfonated macroporous crosslinked, vinylaromatic polymer, the particles having **multimodal** pore-size distribution and a minimum micropore volume of about 0.02 cm.sup.3 /g.

CLPR:
4. A chromatographic column packed with the carbonaceous adsorbent particles which comprise the product of controlled pyrolysis of a polysulfonated macroporous crosslinked, vinylaromatic polymer, the particles having **multimodal** pore-size distribution and a minimum micropore volume of about 0.02 cm.sup.3 /g, wherein the particles are treated, subsequent to pyrolysis, with adsorbable reactive agent.

CCOR:
210/198.2

DOCUMENT-IDENTIFIER: US 5071547 A
TITLE: Column chromatographic column apparatus with switching capability

DEPR:
The fluid conduit assembly or means is comprised of conduit piping connecting
the dual columns, detector, multi-valve arrangement in a similar manner to that
of FIG. 2 with three exceptions. First, the fluid conduit assembly in addition
to conduits 11A through 11L as in FIG. 2, also has conduits 11X through 11Z and
11Z'. Second, there are two valves present in what conceptually can be
referred to as each valve set 12A and 12B of FIG. 2. The third exception is
the presence of an additional valve 42 that isolates the detector from the
pressure of the DCC Apparatus Although valves 12iv and 12v can be indicated as
being inthe valve set 12A of FIG. 2 and valves 12vi and 12vii can be indicated
as being in the valve set 12B of FIG. 2, these valves may exist with
independent identity from these valve sets. In other words these valves may be
just aplurality of valves without valves 12iv and 12v as well as the two valves
12vi and 12vii perform the same function as valves sets 12A and 12B,
respectively. Valve 42 is any multi-port and **multimodal** valve known to those
skilled in the art as are valves 12iv through 12vii, and valve 42 can be
considered a part of the multi-valve arrangement 12. As with the "" Apparatus
of FIG. 2, that of FIG. 2 has each valve with an actuating
connection with
actuating connection 14A as valves 12iv-12vii and 42 are connected by
connections 14J, 14K, 14L, 14M, and 14N, respectively. Valves 12iv and 12v are
connected together for pressurized fluid passage between them by conduit 11X,

and in a similar manner valves 12vi and 12vii are connected by conduit 11Y.

CCOR:
210/198.2

DOCUMENT-IDENTIFIER: US 5019270 A
TITLE: Perfusive chromatography

DEPR:
Broadly, in accordance with the invention, perfusion chromatography is practiced by passing fluids at velocities above a threshold level through a specially designed matrix characterized by a geometry which is bimodal or **multimodal** with respect to its porosity. Perhaps the most fundamental observation relevant to the new procedure is that it is possible to avoid both the loss of capacity characteristic of convection bound systems and the high plate height and bandspreading characteristics of diffusion bound systems. This can be accomplished by forcing chromatography fluids through a matrix having a set of larger pores, such as are defined by the interstices among a bed of particles, and which determine pressure drops and fluid flow velocities through the bed, and a set of pores of smaller diameter, e.g., anisotropic throughpores. The smaller pores permeate the individual particles and serve to deliver chromatography fluids by convection to surface regions within the particle interactive with the solutes in the chromatography fluid.

DEPR:
From the foregoing description many of the basic engineering goals to be pursued in the fabrication of matrix materials suitable for the practice of perfusion chromatography will be apparent to those skilled in the art. Thus, what is needed to practice perfusion chromatography is a matrix which will not crush under pressure having a bimodal or preferably **multimodal** pore structure and as large a surface area per unit volume as possible. The first and second pore sets which give the material its bimodal flow properties

must have mean
diameters relative to each other so as to permit convective flow through both
sets of pores at high V.sub.beds. The provision of subpores in the matrix is
not required to conduct perfusion chromatography but is preferred because of
the inherent increase in surface area per unit volume of matrix material such a
construction provides.

DEPR:
In contrast to the PL 4,000 material, which, with respect to its pore
structure, is **multimodal,** a more ideal perfusive particle might comprise a
plurality of sets of throughpores and subpores of differing mean diameters. A
bimodal pore size distribution can be achieved in such particle by mixing equal
ratios of particles having two discrete pore sizes or by engineering this
feature at the polymerization stage. Ideally, mean diameter ratio between
throughpore subsets would be less than 10, the mean diameter ratio between the
smallest throughpore sets and the subpores would be less than 20, and the mean
diameter ratio between the first pore set, i.e., the intersticies among the
particles making up the matrix, and the largest throughpore subset would be
less than 70, and preferably less than 50. A **multimodal** material might be
produced by agglomerating 500 .ANG. porons to form approximately 1 .mu.m
clusters, which in turn are agglomerated to form 10 .mu.m aggregates, which in
turn may be aggregated to form 100 .mu.m particles. In such a design, the 1
.mu.m clusters would have intersticies of a mean diameter in the vicinity of
a few hundred .ANG.. These would define the [illegible] a very high
surface area. Diffusive transport within these pores would rarely have to
exceed a distance of 0.5 .mu.m or 5,000 .ANG.. Intersticies among the 1 .mu.m
clusters making up the 10 .mu.m aggregates would permit convective flow to feed

the diffusive pores. These would be on the order of 0.3 .mu.m in diameter.
These throughpores, in turn would be fed by larger pores defined by
intersticies among the 10 .mu.m particles making up the 100 .mu.m particles.
These would have a mean diameter on the order of 35 .mu.m.

CCXR:
210/198.2

	Type	L #	Hits	Search Text	DBs	Time Stamp
1	BRS	L1	37759	monolith$3	USPAT	2001/12/18 15:45
2	BRS	L2	1551	210/198.2.ccls.	USPAT	2001/12/18 15:46
3	BRS	L3	40	1 and 2	USPAT	2001/12/18 15:59
4	BRS	L4	942	multimodal	USPAT	2001/12/18 15:59
5	BRS	L5	12	2 and 4	USPAT	2001/12/18 15:59

	Comments	Error Definition	Errors
1			0
2			0
3			0
4			0
5			0

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3	40	monolith$3 and 210/198.2.ccls.	USPAT	2001/12/18 15:59
4	942	multimodal	USPAT	2001/12/18 15:59
5	12	210/198.2.ccls. and multimodal	USPAT	2001/12/18 15:59

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